TIME-FREQUENCY ANALYSIS OF THE HUMAN PHOTOPIC ELECTRORETINOGRAM:

METHOD, NORMATIVE DATA AND CLINICAL APPLICATIONS

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I dedicate this thesis to loved ones, especially...

To my mother and my father for opening my eyes to the world of science, for making me such a perseverant person, and for instilling the importance of hard work and higher education;

To Francesca, my life partner, who always refocused and motivated me when I needed it the most, you are the most wonderful, diverting and powerful person I have ever met!

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Pierre Lachapelle

"If anything, through this epopee I have come to learn that I still have a lot to learn..."

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PREFACE: PART I

1. ABSTRACT

To date, more than 150 years after its discovery, the electroretinogram (ERG) remains the most objective tool to assess retinal function. However, while major improvements have been achieved in ERG recording technologies, the interpretation and the clinical applications of the ERG remain limited as its analysis is still confined to peak time and amplitude measurements. This practice has been proven but limits physiological and clinical interpretation of low voltage ERGs, restricts the number of relevant ERG descriptors and does not consider all morphological aspects of the signal. In an attempt to improve the usefulness and the diagnostic power of the ERG, we set out to investigate the following question: "Can advanced analytical approaches extract additional useful diagnostic or physiological information from the photopic ERG?" To address this question, I isolated more than 10 novel highly reproducible ERG descriptors derived from the discrete wavelet transform (DWT) and demonstrated that these descriptors were physiologically meaningful, diagnostically relevant and usable over a wide range of signal amplitudes and morphologies. Selected DWT descriptors can quantify distinct retinal events and can be independently affected by a given disease process, hence leading to the creation of novel diagnostic classes that better reflect the almost unlimited ways by which the ERG can be altered as a result of normal and pathological conditions. The DWT approach described in the present thesis has the potential to refine the ERG sensitivity and specificity to the point that we might be one day able to identify photopic ERGs that will be characteristic for given disease processes.

2. RÉSUMÉ

Plus de 150 ans après sa découverte, l'électrorétinogramme (ERG) demeure l'outil diagnostique le plus objectif pour évaluer la fonction rétinienne. Cependant, bien que des améliorations majeures aient été réalisées dans les techniques d'enregistrement de l'ERG, l'interprétation et les applications cliniques de l'ERG restent limitées, car son analyse se limite aux mesures de latence et d'amplitude. Bien que cette pratique ait été prouvée, elle limite l'interprétation physiologique et clinique des ERGs de faibles voltages, restreint le nombre de descripteurs pertinents et ne tient pas compte de tous les aspects morphologiques du signal. Dans le but d'améliorer l'utilité et la puissance diagnostique de l'ERG, j'ai entrepris d'étudier la question suivante: « Est-ce l'utilisation d'approches analytiques avancées peuvent extraire des informations diagnostiques ou physiologiques utiles dans l'ERG photopique?» Pour répondre à cette question, j'ai isolé une dizaine de nouveaux descripteurs hautement reproductibles, dérivés de la transformée en ondelettes discrètes (DWT), et j'ai démontré que ces descripteurs avaient une signification physiologique, étaient diagnostiquement pertinents et utilisables sur une large gamme d'amplitudes et de morphologies du signal. Certains descripteurs DWT peuvent quantifier des événements rétiniens distincts et peuvent être affectés de manière indépendante par un processus pathologique donné, ce qui conduit à la création de nouvelles classes de diagnostic qui reflètent mieux les façons quasi illimitées par lesquelles l'ERG peut être modifié en raison de conditions normales et pathologiques. L'approche DWT décrite dans la présente thèse a le potentiel d'affiner la sensibilité et la spécificité de l'ERG au point que nous pourrions un jour identifier des ERGs photopiques qui seront caractéristique de certains processus pathologiques.

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PREFACE: PART II

1. CONTRIBUTION OF AUTHORS ON CO-AUTHORED PAPERS

Because my PhD project was multidisciplinary (ophthalmology, signal processing, and programming), this thesis and the included manuscripts would not have been completed without the precious help, contributions and careful reviewing of several collaborators. In, this section, I would like to detail their contributions of my colleagues as well as my own.

1.1 Manuscript 1 (Chapter II)

Gauvin, M., Lina, J.-M., Lachapelle, P. (2014). *Advance in ERG analysis: From peak time and amplitude to frequency, power, and energy*. BioMed Research International 2014, 246096, 1–11, doi:10.1155/2014/246096.

For this manuscript, the study design was done by Dr. Pierre Lachapelle, Dr. Jean-Marc Lina and me. ERG analyses (Amplitude, peak-time, Fast Fourier Transform, Continuous Wavelet Transform, and Discrete Wavelet Transform), data representation, illustrations and statistical analyses were conducted by me. Data interpretation along with the correction and rewriting of the several iterations of this manuscript were always made in constant collaboration with Dr. Pierre Lachapelle and Dr. Jean-Marc Lina. I have also corrected the manuscript from the reviewer's comments and was actively involved in responding to their remarks.

1.2 Manuscript 2 (Chapter III)

Gauvin, M., Little, J. M., Lina, J.-M., Lachapelle, P. (2015). *Functional decomposition of the human ERG based on the discrete wavelet transform*. Journal of Vision, 15(16):14, 1–22, doi:10.1167/15.16.14.

This second study was designed by Dr. Pierre Lachapelle, Dr. Jean-Marc Lina and me. ERG analyses (Amplitude, peak-time, and Discrete Wavelet Transform), data representation, illustrations and statistical analyses were conducted by me. Dr. John M. Little was the ophthalmologist who followed and confirmed the diagnosis of included patients. Data interpretation along with the correction and rewriting of the several iterations

of this manuscript was always made in constant collaboration with Dr. Pierre Lachapelle and Dr. Jean-Marc Lina. I have also corrected the manuscript from the reviewer's comments and was actively involved in responding to their comments.

1.3 Manuscript 3 (Chapter IV)

Gauvin, M., Chakor, H., Koenekoop, R.K., Little, J. M., Lina, J.-M., Lachapelle, P. (2016). *Witnessing the first sign of Retinitis Pigmentosa onset in the allegedly normal eye of a case of unilateral RP: A 30 year follow-up.* Documenta Ophthalmologica, 132(3):213-229, doi:10.1007/s10633-016-9537-y

For this third manuscript, the study design was done by Dr. Pierre Lachapelle and me. ERG analyses (Amplitude, peak-time, and Discrete Wavelet Transform), funduscopy analyses, visual field analyses, data representation, illustrations and statistical analyses were conducted by me. Dr. Hadi Chakor assisted me with funduscopy analyses. Drs. Robert K Koenekoop and John M. Little were the ophthalmologists who followed and confirmed the diagnosis of included patients. Dr. Koenekoop also performed DNA analyses. Data interpretation along with the corrections and rewriting of the several iterations of this manuscript were always made in constant collaboration with Dr. Pierre Lachapelle and Dr. Jean-Marc Lina. I have also corrected the manuscript from the reviewer's comments and was actively involved in responding to their recommendations.

1.4 Manuscript 4 (Chapter V)

Gauvin, M., Sustar, M., Little, J. M., Brecelj, J., Lina, J.-M, Lachapelle, P. (2016). *Unmasking ERG ON-OFF interactions with the Discrete Wavelet Transform*. Translational Vision Science & Technology. (In Press).

For this fourth manuscript, the study design was done by Dr. Pierre Lachapelle, Dr. Maja Sustar and me. Dr. Maja Sustar and I were equal first authors. I conducted ERG analyses (Amplitude, peak-time, and Discrete Wavelet Transform), data representation, illustrations, and statistical analyses. Data interpretation along with the corrections and rewriting of the several iterations of this manuscript were always made in constant collaboration between Dr. Sustar, Dr. Pierre Lachapelle, Dr. Jean-Marc Lina and me. Dr

Sustar and I also corrected the manuscript from the reviewer's comments and were actively involved in responding to their comments.

1.5 Manuscript 5 (Chapter VI)

Gauvin, M., Dorfman, A., Trang, N., Little, J. M., Koenekoop, R.K., Lina, J.-M., Lachapelle, P. (2016). *Assessing the contribution of the oscillatory potentials to the genesis of the photopic ERG with the Discrete Wavelet Transform*. Biomed Research International. (In Press).

For this last manuscript, the study design was done by Dr. Pierre Lachapelle, Dr. Allison Dorfman and myself. Dr. Allison Dorfman and I were equal first authors. ERG analyses (Amplitude, peak-time, and Discrete Wavelet Transform), data representation, illustrations, and statistical analyses were conducted by me. Dr. Nataly Trang and Mercedes Gauthier assisted us with data retrieval and analyses and with the review of literature. Drs. Robert K Koenekoop and John M. Little were the ophthalmologists who followed and confirmed the diagnosis of included patients. Data interpretation along with the corrections and rewriting of the several iterations of this manuscript were always made in constant collaboration with Dr. Pierre Lachapelle, Dr. Jean-Marc Lina, Dr. Allison Dorfman and me.

CHAPTER I:

GENERAL INTRODUCTION

1. STUDY RATIONALE

The retina is the light-sensitive membrane that ensures light perception and encryption of visual information from the outside world. This neural tissue is located on the internal surface of the eyeball and contains several cell types [photoreceptors (i.e. rods and cones), bipolar, amacrine, horizontal, Muller and ganglion cells], each of them located in specific layers of the retina (Dowling, 1987). When bombarded by photons, such as those emitted by a brief flash of light, the photoreceptors produce graded responses that subsequently trigger a cascade of transient electrochemical events that directly (or indirectly) activates other specialized retinal cells (e.g. horizontal, bipolar, amacrine, Muller and ganglion cells) to ultimately allow the transmission of visual information toward the brain via the optic nerves (Rodieck, 1998). This sequence of transient bioelectrical events also produces negative and positive currents that propagate through the eye from the retina to the cornea. Potential differences can thus be measured, noninvasively, between an active electrode located on the eye (e.g. cornea, sclera, etc.) or close to it and a reference electrode pasted on the skin (e.g. forehead, external canthus, temple, etc.) (Heckenlively, 1991). The stimulation is usually achieved with a white-light flash included in a spherical reflector (i.e. to stimulate the whole retinal surface) also known as a Ganzfeld (i.e. "full-field" in German). The bioelectrical signal thus obtained represents the summed light-evoked responses of all activated retinal cells and is thus usually referred to as a "full-field" flash electroretinogram (ERG). It can be recorded in scotopic (i.e. after dark-adaptation) and photopic (i.e. after light adaptation) conditions to assess the function of the rod (night vision) and cone (day and color vision) pathways, respectively (Heckenlively, 1991). The normal ERG waveform (see Figure 1A) is typically composed of two main waves; 1-a negative deflection (the a-wave) mostly reflecting the hyperpolarization of the photoreceptors, followed by 2-a larger positive wave (the b-wave), which reflects mostly bipolar and Müller cell activities (Hood and Birch, 1990; Newman and Frishman, 1991). The normal function of any retinal cells can be changed as a result of numerous pathological processes (i.e. retinopathies), thus altering some (or all) of the ERG components' amplitude and/or timing (Miyake, 2006). To date, the ERG represents the most objective tool to functionally diagnose, follow the progression or to evaluate the treatment efficiency of a variety of retinal disorders.



Figure 1. Representative example of the photopic ERG signal and of the traditional time domain measurements of the a-wave (Figure 1A, C), b-wave (Figure 1A, C) and filtered (75-500 Hz) oscillatory potentials (OPs; Figure 1B, D) amplitudes (Figure 1A, B) and peak times (1C, D) of the photopic ERG. Amplitude and peak time measurements are illustrated by the thick vertical and horizontal arrows, respectively. Measurements of the a-, b- and i-waves are indicated by the corresponding letter, while that of OP2, OP3 and OP4 are indicated by the corresponding numbers.

Of note, the ERG was first recorded in 1865 when Holmgren discovered that the resting electrical potential of the frog's eye could be altered by a light stimulus (De Rouck, 2006). Twelve years later, in 1877, Dewar independently found that light illumination of the human eye, which was previously dark-adapted (using eye patches) also caused a difference in the resting potential of the cornea (De Rouck, 2006). Of interest, with this discovery, Dewar was not only the first to record an ERG in human, but also the first ever to record a biopotential from a human subject; before the electrocardiogram (Einthoven, 1911) and the electroencephalogram (in 1929; Berger, 1929). Since the first ERG recording (i.e. 150 years ago), the ERG recording technologies were significantly improved. The first ERG signals were recorded with string galvanometers, which were too slow to record rapid changes in the electrical potential (De Rouck, 2006). Nowadays, ERGs are recorded using

fast state-of-the-art amplifiers with analog-to-digital converters and filters, and several responses can easily be averaged to lower the baseline noise level. Indeed, after more than a century of progress, the quality of ERG signal recordings was significantly improved, and the detection of faster components such as the oscillatory potentials (OPs; identified as number 2, 3 and 4 at Figure 1B) was eventually made possible (Cobb and Morton, 1954). While these major improvements in ERG technologies significantly enhanced the quality of ERG recordings, ERG analyses, on the other hand, remained virtually unchanged. In the latest update (2015) of the full-field ERG standard of the International Society for Clinical Electrophysiology (ISCEV), it is suggested to report the amplitude and peak time of the a- and b-waves (McCulloch et al., 2015). This practice of interpreting the ERG using measurements of its two principal components was used almost since the discovery of this signal. Therefore, for more than 100 years, ERG interpretation remained almost entirely limited to amplitude and peak time measurements of the a- and b-waves in the time domain (TD).

Advanced signal processing techniques, such as frequency domain analyses, or more recently, time-frequency domain analyses, have allowed extraction of additional useful information in biomedical signals such as the electroencephalogram (EEG), the electromyogram (EMG), the magnetoencephalogram (MEG), the electrocardiogram (ECG), blood pressure signal, blood-oxygenation-dependent (BOLD) signal, respiratory patterns signal, heart sound signal, and DNA sequences, thus significantly accelerating the rate of significant discoveries and, eventually, improving their diagnostic potential (Addison 2005; Phinyomark et al., 2011; Gadhoumi et al., 2012; Dupuis and Eugene, 2000; Arneodo et al., 1998; Petrosian et al., 2002; Hadjileontiadis and Panas, 1997; Khalil and Duchene, 2000; Marrone et al., 1999). However, these advanced techniques have only been sporadically applied to the ERG. In this thesis, we will demonstrate that performing ERG measurements solely in the time domain might limit the diagnostic power of the ERG signal in both clinical and basic research applications. Given the above, improving ERG analysis using more sophisticated signal processing approaches represents the ultimate motivation of the research project herein described. In this thesis, the following question will be addressed: Can advanced analytical approaches [such as the fast Fourier transform (FFT), continuous wavelet transform (CWT) or discrete wavelet transform (DWT)] extract *additional useful diagnostic or physiological information from the ERG?* In the following pages, the basic background information that is necessary to the understanding of this research project will be presented. The retinal structure and organization are documented in the next section (section 2) while the electroretinogram will be reviewed in the third section (section 3). In the latter section, we will also present and give examples of the current limitations of the traditional ERG analysis approach. Finally, the last sections will present and review alternative analytical approaches for the electroretinogram (section 4), and this thesis' objectives (section 5).

2. THE RETINA

Visual perception starts when incoming light particles (i.e. photons) reach the back of the eye and trigger a cascade of electrochemical signals that the brain can further process and interpret, thus allowing us to see (Rodieck, 1998). The biological structure responsible for this initial step of light perception is the retina. Given its role, the retina is composed of several types of specialized neuronal cells that can detect light, compress spatiotemporal information and efficiently transmit visual information to the brain (Dowling 1987, 2009).

2.1 Retinal Architecture

The retina is the thin light-sensitive tissue that ensures the phototransduction and the initial coding of visual information to be sent to the visual cortex (Kolb, 2003). It is located at the back of the eyes between the vitreous humor and the choroid, and it covers roughly 75% of the internal surface of the eye. The vertebrate retina contains five main neuronal cell types [photoreceptors (rods and cones), bipolar, amacrine, horizontal, and ganglion cells] and one glial cell type (Müller cells) (Bowmaker, 1980; Oyster et al., 1981; Vigh et al., 2000; Razjouyan et al., 2009). These cells are interconnected, and each type is located at a specific layer of the retina or extends through several layers, as illustrated in Figure 2 (Kolb, 1994, 2003; Dowling, 1987, 2009; Tessier-Lavigne, 1991, 2000). As shown in Figure 2, the retinal cell nuclei are located in the three nuclear layers (i.e. outer nuclear layer, and ganglion cell layer) which are linked together by two synaptic layers (i.e. outer and inner plexiform layers) (Kolb, 1994, 2003; Dowling, 1987, 2009).



Figure 2. Representation of the vertebrate retina. Seven cell types are illustrated: Rod, Cone, Horizontal Cell, Bipolar Cell, Müller Cell, Amacrine Cell and Ganglion Cell. Selected layers of the retina are indicated on the left-hand side: Outer Segments (OS), Inner Segments (IS), Outer Nuclear Layer (ONL), Outer Plexiform Layer (OPL), Inner Nuclear Layer (INL), Inner Plexiform Layer (IPL), Ganglion Cell Layer (GCL) and Nerve Fiber Layer (NFL).

2.2 Light-evoked retinal events

In the eye, the light enters through the cornea and is optimally focused on the retina by the lens. The light enters the retina on the side opposite to the photoreceptors. When the light reaches the outer segments (i.e. distal photosensitive part of rods and cones), the photoreceptor cell membranes hyperpolarize (i.e. light-evoked electronegative response), thus producing an electrochemical response (Rodieck, 1998). The latter is the first necessary step that leads to vision (Ripps, 2010). When photoreceptors hyperpolarize, they activate the second layer of cells (i.e. inner nuclear layer; INL at Figure 2) by reducing the release of the neurotransmitters (glutamate) in the synaptic layer known as the outer

plexiform layer (OPL at Figure 2) (Djamgoz et al., 1995; Rodieck, 1998; Hack et al., 1999). The INL contains bipolar cells (ON and OFF), amacrine cells, and horizontal cells as well as Müller cells (glia) (Dowling, 1987, 2009; Bringman et al., 2006). In the OPL, bipolar cell synapses receive direct inputs from the rods and cones, but also indirect ones from the horizontal cells (Dacey, 1999; Razjouyan et al., 2009; Dowling, 2009). Rods mostly connect to ON bipolar cells while cones connect to both ON and OFF bipolar cells (Forrester et al., 2002). Given that glutamate can be excitatory and inhibitory, it has a different effect depending on which glutamate receptors (metabotropic or ionotropic) upon which it is released (or not released) (Tessier-Lavigne, 1991, 2000). For example, when cones stop releasing glutamate (in light), it causes the cone ON bipolar cells to lose their inhibition and to become active (depolarized; i.e. light-evoked electropositive response), while the cone OFF bipolar cells lose their excitation (hyperpolarize) and become inactivated (Kolb, 1994; 2003; Dowling, 2009; Tessier-Lavigne, 1991, 2000). In short, ON bipolar cells are activated at the onset of a light stimulus and OFF bipolar cells are activated at the offset of light. These properties of the so-called "ON and OFF pathways" result in the detection of light and dark edges and thus in contrast perception (Margolis et al., 2010; Liang and Freed, 2010). Similarly, the activation/deactivation of bipolar cells causes variation in the amount of neurotransmitters that they release in the second synaptic layer (i.e. the inner plexiform layer, or IPL); in turn, these neurotransmitters directly activate the ganglion cells (located in the ganglion cell layer: GCL), or indirectly through amacrine cells (Stone et al., 1987; Do-Nascimento 1991). Like horizontal cells, amacrine cells introduce lateral inhibition, giving rise to center-surround inhibition that creates a unique receptive field for each ganglion cell (Vigh et al., 2000). Retinal ganglion cells connected to ON or OFF bipolar cells maintain a similar ON and OFF configuration (Dowling, 2009; Margolis et al., 2010). Finally, when activated, the ganglion cells can produce action potentials (when depolarized above the threshold) that can rapidly propagate in their myelinated axons (Dowling, 2009). These cells thus initiate the last step of retinal light perception: sending visual information to the brain (Rodieck, 1998). The axons of the ganglion cells (which form the nerve fiber layer) leave the retina by forming the optic nerve and convey the neural signals generated by the retina to the brain.

To date, clinical examination of the retina with the ophthalmoscope or with specialized fundus cameras remains one of the most widely used ophthalmologic/retinal diagnostic tools, but it does not always reveal diagnostic signs of the suspected retinopathy on the retina (Nettleship, 1908, 1914; Pearlman, 1976). Consequently, the electroretinogram, which is the topic of the next section, is often considered as an alternative objective diagnosis tool that complements retinal imaging.

3. THE ELECTRORETINOGRAM

As above-mentioned, when stimulated by light, the photoreceptors produce electrochemical signals that are subsequently processed in the other retinal layers to allow the pre-processing of light information before sending it to the brain (Tessier-Lavigne 1991, 2000; Rodieck, 1998; Dowling, 2009). This information is ultimately destined to the brain, but the sequence of transient electrochemical events that happens at the retinal level can be recorded, non-invasively, using an electrode located on the eye (i.e. cornea or conjunctival sac) or close to it (i.e. lower/upper lids, external/internal canthi, etc.) (McCulloch et al., 2015). The graphic representation of the light-evoked voltage changes thus obtained is called the electroretinogram.

3.1 <u>History</u>

In 1865, Holmgren discovered that the resting electrical potential of the frog's eye could be altered by light (De Rouck, 2006). Twelve years later, in 1877, Dewar independently found that light illumination of the human eye, which was previously dark-adapted (using eye patches) caused a difference in the resting potential of the cornea (Dewar, 1877). This light-evoked biopotential was later termed the electroretinogram, often abbreviated as ERG. Later in 1903, Gotch reported, for the first time, that the frog ERG, evoked to brief flashes of light, was formed of two waves: a first negative wave of low amplitude and a second positive wave of larger amplitude (Gotch, 1903). Einthoven and Jolly (1908) went further and suggested that the ERG signal could even be decomposed in three distinct waves: a negative wave (a-wave) and a positive wave (b-wave) followed by a slower positive wave (c-wave) (Einthoven and Jolly, 1908). Later (1933), Granit published an important study conducted on cats, which largely contributed to the

understanding of the ERG. In this study, Granit showed that the ERG was composed of three distinct components that he isolated and termed PI, PII, and PIII, according to their respective order of disappearance as the level of anesthesia is increased (Granit, 1933). These processes were shown to be associated with the previously identified c-, b- and a-waves, respectively. Subsequently, it was rapidly demonstrated that these waves originated from distinct retinal structures and relationships between the stimulus intensity and the amplitude of the response were documented. As we will see below, other components, such as the oscillatory potentials (OPs), d-wave and i-wave, were also considered/discovered over the years.

3.2 The full-field photopic ERG

To perform a global functional evaluation of the whole retina, the ERG is evoked to a homogeneously diffused light stimulus. The uniform stimulation of the entire retinal surface is usually achieved using a Ganzfeld (i.e. full-field) stimulator. It simply consists of a flash incorporated into a spherical reflector. Evoked as such, the so-called "full-field" ERG represents a pan-retinal response evoked by a flash stimulus. The ERG can also be evoked by more complex and/or structured stimuli (e.g. multifocal and pattern ERGs) (Hood et al., 2003, 2012; Wanger and Persson, 1983), but this thesis will be limited to the full-field ERG given its widespread use in both basic research and clinical diagnosis. Of note, it is also possible to control certain acquisition parameters to influence the desired ERG response. For example, when recording the ERG against a rod-desensitizing background (i.e. projected by the Ganzfeld), we can more selectively evaluate the function of the cone pathway. In summary, the ERG can be recorded in photopic (i.e. light adapted) and scotopic (dark-adapted) conditions to evaluate the specific function of the cone and rod pathways, respectively.

Although advanced analytical approaches could theoretically be applied to any type of ERG signals, in the framework of this thesis, we mainly focused our studies on the photopic ERG for several reasons. Firstly, the cone pathway is the most valuable pathway for vision given that it allows color vision, contrast perception and visual acuity (Dacey, 1999; Tessier-Lavigne, 1991, 2000; Dobelle et al., 1996). Secondly, when evoked to different stimulus intensities, the peak time of its major components (e.g. b-wave) vary

little (b-wave implicit time between 25 and 35 ms) compared to the peak time shift measured in the scotopic condition (b-wave implicit time ranges from 40 and 125 ms) (Garon et al., 2010; Velten et al., 2001). This smaller peak time shift significantly eases the selection of relevant time-locked descriptors. Thirdly, the photopic response is purely conedriven, thus reducing the biological variation that could emerge from complex interactions between the rod and cone pathways in the normal and diseased retina. Fourthly, the photopic ERG is often the only sign of retinal function remaining from patients affected with severe degenerative retinopathies (such as retinitis pigmentosa: RP), thus offering the longest time window to monitor patients (Rispoli et al., 1994). Fifthly, we had in our possession a databank of close to 40 years of clinical ERGs obtained from patients affected by a variety of retinal disorders, and while our recording standard of the scotopic ERG changed throughout the years, our photopic ERG standard always remained relatively similar. We also limited our study to the photopic ERG for the uniqueness of its luminanceresponse function (the so-called Photopic Hill; see section 3.4.3), with its four typical phases (i.e., ascent, maximal value, descent, and final plateau phases) and ensuing different cone ERG morphologies (Garon et al., 2010, 2014; Hamilton et al., 2007; Kondo et al., 2000; Rufiange et al., 2002, 2003, 2005; Ueno et al., 2004; Wali and Leguire, 1992). In summary, given all of the above, this thesis will be limited to the photopic ERG to improve our understanding of the cone ERG and to refine the analytical methods used to analyse it.

3.2.1 Photopic ERG components and their origin

The photopic ERG is typically composed of a negative deflection (the a-wave), followed by a larger positive wave (the b-wave). On the ascending limb of the b-wave are also seen small high-frequency oscillations called the oscillatory potentials (OPs) (Hébert and Lachapelle 2003). These main components can be modulated by several factors, such as the size of the pupils (Gagné et al., 2010), the intensity (Wali and Leguire, 1992), duration (Sustar et al., 2006, 2008), frequency (Shuang et al, 1995) and color (Rufiange et al., 2005) of the stimulus, the rod-desensitizing background intensity and color (Rangaswamy et al, 2007), as well as the amount of light and/or dark-adaptation (Garon et al., 2010). For more than a century, scientists have been thoughtfully investigating the genesis and the origin of the ERG components, and as a result, our understanding of the

photopic ERG was significantly enriched. This section briefly summarizes our current knowledge of the generators (i.e. retinal cells) of the a-wave, b-wave, and OPs, as well as that of other ERG components (e.g. d-wave).

3.2.1.1 The a-wave

The a-wave (Figure 1A) is the initial negative deflection of the ERG that closely follows the onset of the stimulus and that immediately precedes the b-wave. It is now widely accepted that the photopic a-wave genesis is attributed mainly to the light-evoked hyperpolarization of the cone photoreceptors (as rods are saturated in light-adapted conditions), which in turn, explains its negative (hyperpolarization) polarity (Penn and Hagins, 1969; Brown, 1968; Armington, 1974; Hood and Birch 1990). In the context of this thesis, the color of the flash stimulus was always white (i.e. a combination of many wavelengths) and thus, our a-waves originated from the summed responses of the three types of cones [short (blue), medium (green) and long (red) wavelength cones]. Of interest, several pharmacological blockage studies have demonstrated that the photoreceptors are not the sole generators of the a-wave, and that postreceptoral cells also contribute to this ERG component. For example, in primates, it was shown that blocking the synaptic communication from photoreceptors to second order neurons of the inner retina (with intravitreal injections of piperidine-dicarboxylic acid: PDA) results in a significant reduction of the a-wave amplitude (Bush and Sieving, 1994). Given that PDA primarily blocks the response of horizontal cells and hyperpolarizing (i.e. OFF) bipolar cells, slight contributions of these cells to the a-wave cannot be excluded. Similarly, injections of cobalt or aspartate, which are known to suppress all postreceptoral responses, also result in a significant decrease of the a-wave amplitude (Dong et al., 2014). Clearly, the inner retina also has a minor role in shaping the a-wave of the ERG.

3.2.1.2 The b-wave

Following the a-wave, a larger wave of positive polarity, named the b-wave (Figure 1A), can be seen. Of interest, the b-wave is generally the most prominent component of the ERG and therefore, its amplitude is one of the most widely quantified parameters of the ERG. Of note, in certain conditions, such as when a pure-rod response is recorded, the b-

wave can be recorded even if the ERG does not present with a visible a-wave (McCulloch et al., 2015). Thus, in scotopic conditions, the b-wave does not always necessarily follow an a-wave. For decades, the b-wave has been presented as the result of almost synchronized interaction of second-order neuronal cells with glial cells; the so-called bipolar/Müller cell complex (Miller and Dowling, 1970; Newman and Frishman, 1991; Sieving et al., 1994). This generally accepted concept emerged from years of experimental research. Initially, it was found that the maximal b-wave amplitude was reached at the level of the inner nuclear layer (INL at Figure 2) and it was hence suggested that the bipolar cells were the main generator of the b-wave (Brown and Wiesel, 1961). Less than a decade later, refined intracellular recordings revealed the importance of Müller cells in the genesis of the bwave and an elegant model of current source density was proposed (Faber, 1969, Miller and Dowling, 1970). Briefly, when bipolar cells are activated (secondly to the light-evoked photoreceptor hyperpolarization), the extracellular concentration of potassium ions (K+)increases in the distal part of the inner retina. Given that the Müller cell membrane is highly and selectively permeable to K+ (Kuffler, 1967; Newman, 1985), there is a subsequent influx of K+ at the distal part of the Müller cells (resulting in a depolarization of the Müller cell), and subsequently, an outflow of these K+ ions at the more proximal part of the Müller cell, namely in the vitreous (Faber, 1969, Miller and Dowling, 1970; Dick and Miller, 1978; Newman, 1979; Newman and Odette, 1984). This sinking of ionic fluxes thus created by the Müller cells is believed to generate a transretinal potential which appears as the b-wave in the corneal ERG. Supportive of the latter-termed *K*+ *hypothesis*, work on the mudpuppy demonstrated that the morphology and timing of the intracellular potential of the Müller cells, in response to a light stimulus, was highly similar to that of the corneal b-wave evoked to the same stimulus (Miller and Dowling, 1970). The K+ hypothesis was further confirmed in other studies, such as with intravitreal injections of K+, which create potential differences of the Müller cell membrane that resembled that of the corneal b-wave (Fujimoto and Tomita, 1981; Yanagida and Tomita, 1982), or by intraocular injections of DL- α -amino adipate, a toxin that specifically affects the Müller cell, which result in a loss of the b-wave, while other components (e.g. the a-wave) remain unaffected (Bonaventure et al., 1981). Based on the above, it appears that the Müller cells are the main retinal generator of the b-wave; but given that the change in the electrical potential of these glial

cells is the result of the absorbed extracellular K+, their activation is only a by-product of the activation of primary neuronal cells (e.g. the bipolar cells), which modify the extracellular K+ concentration in the first place (Dick and Miller, 1985; Dick et al., 1985). Indeed, as many studies have shown, without the primary activation of neuronal bipolar cells, there is no subsequent depolarization of the Müller cells and thus no b-wave. In fact, the Müller cells do not even play an active role in the transmission of the light information through the retina and to the brain (Newman and Reichenbach, 1996). In that matter, the bipolar cells are the first contributor to the building of the b-wave. Attesting to the latter, following intraocular administration of 2-amino-4-phosphonobutyric acid (APB), which selectively abolishes ON bipolar cell function (Slaughter and Miller, 1985), the b-wave of the ERG is almost completely eliminated (Stockton and Slaughter, 1989; Gurevich and Slaughter, 1993). Similarly, intravitreal injections of piperidine-dicarboxylic acid demonstrated that the OFF-bipolar cells were also involved in shaping the b-wave (Bush and Sieving, 1994). With the latter discovery, Sieving introduced the push-pull concept, where both the ON- and OFF-bipolar cells shape the b-wave. It was also suggested that this interaction was stimulus intensity-dependent, with the ON-bipolar cells "pushing" the b-wave up in the ascending phase of the Photopic Hill and the OFF-bipolar cells "pulling" down the b-wave in its descent (Rufiange et al., 2002, 2003). In summary, the bipolar/Müller cell complex, which states that the b-wave mainly results from contributions of bipolar and Müller cell activities remain widely accepted.

3.2.1.3 The oscillatory potentials

In the earliest ERG recordings, only the above-defined low-frequency a- and bwaves were identifiable (Einthoven and Jolly, 1908). Subsequently, with the development of more advanced recording systems (e.g. those that were able to record faster potentials), higher frequency components began to appear on the ERG. As early as 1954, lowamplitude high-frequency oscillations were observed on the ascending limb of the b-wave (Cobb and Morton, 1954). In 1962, Yonemura and colleagues were the first to call these high-frequency oscillations the *oscillatory potentials* (OPs) (Yonemura et al., 1962a). Given that the OPs oscillate between 70-200 Hz, they can be extracted by rising the lowfrequency cutoff of the recording bandwidth. This traditional technique (i.e. bandpass

filtering) removes (or at least attenuates) the low-frequency components (i.e. a- and bwaves) from the ERG signal, thus permitting a better visualization and quantification of the OPs. For example, while the broadband ERG shown in Figure 1A was recorded between 1 Hz (low cutoff) and 500 Hz (high cutoff), the OPs (Figure 1B) were extracted by rising the low cutoff to 75 Hz. In the case of a light-adapted retina (i.e. photopic condition) evoked to the suprathreshold stimulus intensity [i.e. an optimal stimulus previously documented by us (Lachapelle et al., 2001)], three OPs are easily identified; OP1-2, OP3, and the long latency OP4, which are identified as 2, 3 and 4 at Figure 1B. To date, despite a substantial amount of studies published on the OPs, there is still no clear consensus on the cellular origin or on the mechanisms of OPs genesis. However, it is widely accepted that OPs are generated in the inner retina (Brown, 1968; Ogden, 1973; Miller and Dowling, 1970; Wachtmeister 1978; Heynen et al., 1985). Effectively, studies have shown that conditions that create an ischemic incident specifically limited to the inner retina (e.g. central retinal artery occlusion) eliminate both the b-wave and OPs of the ERG. demonstrating that the OPs are dependent on the inner retinal circulation and thus most probably generated in the latter layer (Brown, 1968). Microelectrode studies conducted at different depths of the primate retina confirmed that the OPs were generated in the inner retina (Ogden, 1973). In the latter study, the bipolar, amacrine and ganglion cells were identified as the most probable generators of OPs in the inner retina (Ogden, 1973). In contrast, it was shown that due to their slow time-constant membrane, the Müller cells could not generate high-frequency fluctuations and they were ruled out as OP generators (Ogden, 1973). Later, new findings suggested that different OPs were generated at different depths of the retina; the short-latency OPs would be generated more proximally and the long-latency OPs more distally in the retina, suggesting that each OP would have a specific cellular origin (Wachtmeister and Dowling, 1978). Several studies even suggested that the short-latency early OPs originate from the ON (e.g. ON bipolar cells) retinal pathway and conversely, that the long-latency OPs originate from the OFF (e.g. OFF bipolar cells) retinal pathway (Ogden, 1973; Nelson et al., 1978; Kojima and Zrenner, 1978). Similarly, patients affected with congenital stationary night blindness (CSNB), a condition that is known to specifically disturb the ON-pathway (Langrová et al., 2002; Miyake et al., 1987; Quigley et al., 1996; Bech-Hansen et al., 2000; Dryja et al., 2005; Gregg et al., 2007; Pusch et al., 2000), present with a specific abolition of short-latency OPs (Lachapelle et al., 1983). Given the above findings, the bipolar cells appear to be the most probable generators of the OPs. However, the contribution of other cells (e.g. horizontal, amacrine, and ganglion cells) cannot be fully excluded. Of interest, several studies suggest that the retinal generators of the OPs are independent of those involved in the genesis of the slower a- and b-waves (Yonemura et al. 1962b; Speros and Price 1981; Hamasaki and Maguire, 1985). For example, in some retinopathies (such as in diabetic retinopathy or central retinal vein occlusion), the OPs appeared to be selectively more affected compared to the relatively better preserved a- and b-waves (Yonemura et al., 1962b; Takei et al. 1993; Kizawa et al., 2006). Contrasting with the above are previous findings which demonstrated that the attenuation of specific OPs results in ERG waveforms with predictable (and reproducible) morphologies (Lachapelle, 1987, 1990, 1994; Lachapelle and Molotchnikoff, 1986; Lachapelle et al., 1983, 1998; Heckenlively, 1983; Miyake, 1997). More specifically, the light-adapted ERG of patients affected with CSNB is frequently reported as having a square-wave-like a-wave and a truncated b-wave morphology, which is thought to originate from the absence of OP2 and OP3 on the ascending limb of the b-wave, while OP4 remains unaffected (Lachapelle et al., 1983). Conversely, patients affected with congenital postreceptoral cone pathway anomaly (CPCPA; an OFF-pathway anomaly) present with an attenuated b-wave due to an attenuation and delay in OP3 and OP4, while OP2 remains intact (Garon et al., 2014; Lachapelle et al., 1998). The above findings could suggest that a close relationship exists between the OPs and the development (and morphology) of the b-wave, where each OP could contribute to a specific step in the genesis of the b-wave (Lachapelle et al., 1983).

3.2.1.4 Other components

The push-pull concept introduced by Sieving et al. suggests that the genesis of the photopic ERG b-wave results from a balanced contribution of the ON- and OFF-pathways (Sieving et al., 1994). Indeed, when the photopic ERG is evoked in response to a flash of short-duration (e.g. < 5 ms), the activity of the ON- and OFF-pathways are merged to form the b-wave we record. In contrast, when a stimulus of longer duration (e.g. > 150 ms) is used, distinct responses can be recorded at both the stimulus onset and offset, respectively.

The latter are termed the ON and OFF response, respectively. The long-flash ERG waveform presents with a negative a-wave, followed by a positive b-wave representing the ON response and lastly a positive d-wave representing the OFF response (Sieving, 1993; Sieving et al., 1994; Ueno et al., 2004, 2006; Sustar et al., 2006, 2008). Studies in non-human primates revealed that the major part of the long-flash b-wave originates from the ON-bipolar cells activity (Sieving et al., 1994), while the source of the d-wave was related to the OFF-bipolar cell activity (Ueno et al., 2006). Therefore, it appears that the long-flash ERG allows a more selective evaluation of the retinal ON (b-wave; ON response) versus OFF (d-wave; OFF response) bipolar cell activity. The latter can be helpful for the evaluation of the inner retinal function in various retinal dystrophies (Miyake et al., 1987; Alexander et al., 1992; Sieving, 1993; Ruether and Kellner, 1998).

3.3 The ERG in diseased retina

Depending on the nature of the disease, retinal disorders may affect the amplitude and/or the peak time of the photopic and/or scotopic ERGs. Analysis of scotopic and photopic ERGs combined with other clinical outcomes, such as the appearance of the fundi, visual fields areas, patient's symptoms, etc., will help the clinician to determine (or confirm) the appropriate diagnosis. The section below provides an overview of selected retinopathies that were studied during this PhD project.

3.3.1 Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a progressive degenerative retinal disorder. While RP incidence is low (1 in 4000), it is nonetheless the most common form of inherited retinal degeneration worldwide (Sharma and Ehinger, 1999). RP patients usually present with night-blindness and visual field (VF) constriction (ultimately to tunnel vision) (Madreperla et al., 1990; Marmor, 1991; Grover et al., 1997; Birch, 2006). Retinal fundus examination eventually reveals progressive narrowing of retinal blood vessels, bone spicule (pigmentary deposits) formations and pallor of the optic nerve head (Godel and Regenbogen, 1976; Berson, 1993; Akyol et al., 1995; Li et al., 1995). Depending on the stage and severity of the disease process, the ERG responses can be unaffected to abolished. In most cases of RP, a gradual reduction in the amplitude of the scotopic ERG
is first noted and followed, at a later stage, by a decrease of the photopic ERG as well. At the end-stage of the disease process, both the rod and cone ERGs are extinguished (Berson, 1993; Birch, 2006). Bilateral blindness may happen in the most severe RP cases. Given the large variations in inheritance, severity, and symptoms seen among patients, it might be appropriate to recognize RP as the *RP spectrum* rather than as the RP disease (as several conditions lead to RP-like degenerations). For example, Usher syndrome and Leber congenital amaurosis (LCA) result in some forms of RP. The various forms of RP result from genetic mutations scattered among the different chromosomes. To date, there are at least 45 known gene mutations which account for 60% of RP patients (Hartong and Berson, 2006). As for the inheritance mechanism, it is estimated that the frequency of transmission modes is 19 % autosomal dominant, 8% X-linked, 19 % autosomal recessive, 46% isolated and 8% unknown (Bunker, 1984; Frishman et al., 1985, 1986). While some treatments are currently under investigation, there is no cure for RP yet. Meanwhile, retinal implants can be used to restore some vision (Zrenner, 2002) and intra-retinal injections of stem cells might also be tested in humans in the near future (Mohand-Said et al., 1997).

3.3.2 Congenital stationary night blindness

Congenital stationary night blindness is another retinal disorder which is mostly characterized by nyctalopia (i.e. difficulty to see in the dark). As the name of the disease suggests, this condition is stationary (i.e. non-progressive) and present from birth (i.e. congenital). Therefore, in contrast to RP, CSNB generally does not progress with time nor result in blindness. CSNB patients have normal retinal fundi, but patients often have a slightly reduced visual acuity, often associated with the frequently accompanying moderate to severe myopia. There are two types of CSNB. The complete form (CSNB-1) is accompanied by moderate to severe myopia and is electrophysiologically characterized by the complete absence of rod b-waves (thus explaining the nyctalopia) and the less affected photopic responses. In the incomplete form (CSNB-2), patients present with moderate myopia and abnormal scotopic and photopic ERGs (Miyake et al., 1987; Bech-Hansen et al., 1998). CSNB transmission was shown to be either X-linked (and frequently associated with a mutation in the CACNA1F gene) or autosomal recessive (and frequently resulting from a mutation in the CABP4 gene) (Hoda et al., 2005; Zeitz et al., 2006). Furthermore,

the retinal anomaly of patients affected with CSNB is known to specifically lie on the ONpathway [based on electroretinographic (Langrova et al., 2002; Miyake et al., 1987; Quigley et al., 1996) and molecular (Bech-Hansen et al., 2000; Dryja et al., 2005; Gregg et al., 2007; Pusch et al., 2000) findings]. For example, long-flash ERG studies revealed an abnormal or undetectable ON response (b-wave) and a normal OFF response (d-wave), therefore suggesting that CSNB-1 would result from a synaptic connectivity defect between the photoreceptors and ON-bipolar cells (Miyake et al., 1987; Quigley et al., 1996; Langrova et al., 2002). Complementarily, genetic/molecular studies have recently shown that the X-linked and autosomal recessive form of CSNB result from mutations of the NYX and GRM6 genes, respectively (Pusch et al., 2000; Bech-Hansen et al., 2000; Dryja et al., 2005; Zeitz et al., 2005). These genes have been shown to encode nyctalopin (NYX gene) and the glutamate receptor mGluR6 (GRM6 gene). The latter were previously associated with synaptic transmission between photoreceptors (cones and rods) and ON-bipolar cells (Morgans et al., 2006; Gregg et al., 2007). Finally, animal models of CSNB-1 confirmed that nyctalopin and mGluR6 were implicated in the synaptic transmission between photoreceptors and ON-bipolar cells (Bahadori et al., 2006; Pinto et al., 2007). Of note, the photopic ERG of patients affected with CSNB-1 is usually of normal amplitude and peak time. Despite these normal features, the morphology of the CSNB-1 ERG show, upon visual inspection, a pathognomonic feature. As aforementioned, the photopic CSNB-1 ERG is therefore often qualitatively reported as having a square-wave-like a-wave and truncated b-wave morphology (Heckenlively et al., 1983; Lachapelle et al., 1983; Miyake et al., 1997).

3.3.3 Congenital postreceptoral cone pathway anomaly

Of interest, there is another retinal condition that is known to alter the morphology of the photopic ERG waveform. This interesting retinal anomaly was, to our knowledge, first reported by Lachapelle et al., (1998) and described as a form of congenital postreceptoral cone pathway anomaly, later termed CPCPA (Garon et al., 2014). CPCPA patients have normal visual fields, normal fundus appearance, and normal fluorescein angiograms, but have a reduced visual acuity and a red-green color defect, with no significant interocular differences for any tests (Lachapelle et al., 1998). In contrast to CSNB, no genetic or molecular study has been conducted on CPCPA patients yet, but the condition is believed to be transmitted via an autosomal recessive manner (Lachapelle et al., 1998). The CPCPA ERG waveform has been studied in detail (Lachapelle et al., 1998; Garon et al., 2014). The dark-adapted ERG presents with normal a-wave amplitude and peak time while the amplitude of the b-wave is minimally, but significantly, attenuated with normal timing. Similar to CSNB-1, photopic ERG analyses of CPCPA patients reveals normal cone function (i.e. normal a-wave amplitude), but a striking change of the ERG waveform morphology upon visual inspection. The photopic b-wave is wider (i.e. less sharp) and attenuated in amplitude with a truncated appearance (almost negative morphology). Complementary to CSNB patients, who are lacking OP2 and OP3, CPCPA patients present with normal OP2 and OP3 amplitude, but a severely attenuated OP4. Therefore, the functional anomaly of patients affected with CPCPA is believed to lie in the OFF-pathway (Garon et al., 2014; Lachapelle et al., 1998). It is still unclear if CPCPA is stationary.

3.3.4 Other retinal and non-retinal conditions

While the above retinal conditions were more extensively studied throughout this thesis, other retinal conditions were also explored, albeit to a lesser extent. For example, diabetic retinopathy (DR), which is a relatively common complication of diabetes. In most cases, DR remains asymptomatic until it has caused significant damage to the retina, resulting in a progressive decrease in visual function (Tzekov, 2015). The ERG represents an objective tool to detect early DR changes in retinal function. For example, the 30 Hz flicker ERG amplitude and peak time was previously associated with the severity of DR (Tahara et al., 1993; Jansson et al., 2015). Similarly, previous studies have shown that in DR, the OPs of the full-field ERG are selectively more affected compared to the relatively better preserved a- and b-waves (Yonemura et al., 1962b; Kizawa et al., 2006). Likewise, central retinal vein occlusion (CRVO) is another fairly common retinal vascular disorder affecting the ERG. The most severe type of CRVO is the ischemic form. Ischemic CRVO patients present with hemorrhages at fundus examination, as well as moderate to severe visual loss. The poor perfusion of the inner retina results in electroretinographic changes (Takei et al. 1993; Larsson et al., 2000). Similar to DR, the full-field ERG of CRVO

patients also presents with selectively more affected OPs (Takei et al., 1993). Likewise, the 30Hz flicker ERG is also affected (Larsson et al., 2000). Moreover, another rare X-linked degenerative disorder, called choroideremia (CHM), is clinically characterized by the atrophy of the choroid, of the retinal pigment epithelium and of the retina (Mura et al., 2007). CHM eventually leads to a very typical deterioration of the retinal fundi characterized by a hypopigmented appearance and severely attenuated retinal blood vessels diameters (Mura et al., 2007). The ERG of CHM affected patients is abnormal and indicates a global photoreceptor dysfunction (Coussa and Traboulsi, 2012). The rod ERG is usually more affected, but both the photopic a-wave and b-wave can be severely reduced in amplitude. Finally, hundreds of other conditions and diseases are known to have different effects on the ERG waveform.

3.4 Traditional photopic ERG analysis

As indicated in the above section, the ERG is used to study a variety of retinal conditions or even to explore the physiology of the retina in response to environmental changes. Once ERG recordings are completed, the resulting signals are reported on a graph showing the time course (generally reported in milliseconds) of the signal's amplitude (commonly reported in microvolts) or archived for future analysis. Given that the dependent variable (amplitude) is displayed as a function of time (i.e. independent variable), subsequent measurements done on these graphs are by definition achieved in the time domain (TD). In clinics, the ERG analysis is commonly based on the amplitude and peak time of the a- and b-waves evoked in both light and dark adaptation state. The functional diagnosis of retinopathies is usually confirmed once the amplitude and/or the peak time of the a- and/or b-waves are significantly changed (Kaye and Harding, 1988; McCulloch et al., 2015). In this section, the traditional measurements of the various photopic ERG components will be presented given that they were included and used as the gold standard in this thesis.

3.4.1 Amplitude measurements

The amplitude of the a-wave is measured from the prestimulus baseline amplitude to the most negative trough of the ERG that precedes the b-wave (Figure 1A). Similarly, as shown in Figure 1A, the b-wave is measured from the trough of the a-wave to the most positive peak that follows the a-wave (McCulloch et al., 2015). Photopic OPs are less often considered, most probably because their analysis is not yet part of the ISCEV standard. Under photopic condition evoked to a suprathreshold stimulus intensity, there are generally 3 main OPs (see Figure 1B). Photopic OP amplitudes are measured from the trough to the peak of each OP (Figure 1B). Additionally, the amplitude of the d-wave is measured from the beginning of the d-wave to the most positive peak of the d-wave.

3.4.2 Peak time measurements

As illustrated in Figure 1C, the peak time of the a- and b-waves is measured from the flash onset to the through/peak of the a- and b-waves (McCulloch et al., 2015). Furthermore, as shown in Figure 1D, the peak time of each OP is measured from flash onset to the peak of each OP and the peak time of the d-wave is measured from the offset of the stimulus to the peak of the d-wave.

3.4.3 The luminance-response function of the photopic ERG

The amplitude and peak time of the ERG depend on various stimulus parameters (e.g. intensity, color, and duration of the flash and/or intensity and color of the background light). The relationship between the amplitude of the ERG and the flash luminance has been extensively studied. For example, it is well-known that progressively brighter flashes almost linearly increase the amplitude of both the scotopic and photopic a-waves (Naka and Rushton, 1966; Hébert and Lachapelle, 2003; Rufiange et al., 2002). Similarly, the scotopic b-wave amplitude gradually augments with progressively brighter intensities, following a sigmoidal luminance-response (LR) function, which can be fitted using the Naka-Rushton equation (Naka and Rushton, 1966; Rufiange et al., 2002). In contrast, the LR function of the photopic ERG b-wave is unique in that, with a gradual increase in the stimulus intensity, the amplitude of the b-wave increases, reaches a maximal value, and then decreases before reaching a plateau with the brightest intensities. Because of this particular hill shape, Wali and Leguire (1992) termed the LR function of the cone ERG the *photopic hill* (PH). This phenomenon was extensively explored (Garon et al., 2010, 2014; Rufiange et al., 2002, 2003, 2005; Hamilton et al., 2007; Kondo et al., 2000; Ueno et al.,

2004). While the Naka–Rushton function can easily be used to fit and study the LR function of the scotopic ERG (Rufiange et al., 2002), the rather unique presentation of the cone LR function complicates this kind of curve fitting analyses. It was initially suggested to use a set of 7 easily identifiable and reproducible parameters to analyse the cone LR function (Rufiange et al., 2003). Later, Hamilton and colleagues successfully fitted the photopic hill with an equation that combined a Gaussian and a logistic growth function (Hamilton et al., 2007). They also noted an almost nonexistent contribution of the logistic component in patients with CSNB-1 and suggested that the logistic growth and the Gaussian functions would reflect the ON and OFF retinal responses, respectively (Hamilton et al., 2007). By studying the LR function of patients that are known to have an abolished ON (CSNB-1) or OFF (CPCPA) pathway, it was later confirmed that the Gaussian and logistic growth functions respectively reflected the OFF and ON retinal responses (Garon et al., 2014). The above study confirmed that the photopic hill fitted with a Gaussian/Logistic function could be used to decompose the PH in ON and OFF components without the use of long duration flashes.

3.5 Limitations of traditional time domain analyses of the short-flash ERG

In this section, we will illustrate why solely performing ERG measurements in the time domain might theoretically limit the diagnostic power and the usefulness of the ERG signal in both clinical and basic research applications.

3.5.1 Quantification of ERG morphology

To date, using the ERG descriptors offered by the time domain, functional diagnosis of retinopathies is usually confirmed once the amplitude and/or the latency of the ERG is significantly changed (Kaye and Harding, 1988; McCulloch et al., 2015). The latter thus lead to two main pathological ERG classes: 1-reduced ERGs with normal peak time and 2- reduced ERGs with delayed peak time (although pathological ERGs might also be, albeit more rarely, of normal or increased amplitude with or without delayed peak time). On the other hand, visual inspection of certain pathological ERGs reveals that, besides amplitude and/or latency changes, retinopathies can also markedly modify the morphology of the ERG; a feature of ERG analysis that remains underexploited. The concept of "ERG

morphology" is better illustrated in Figure 3, where pathological ERGs of reduced b-wave amplitude with and without peak time delays (blue and green tracings, respectively) are compared to a normal ERG (black tracings) evoked at identical stimulus intensity.



Figure 3. Pathological ERGs of low amplitude with normal timing (Figure 3A, green tracings) or of low amplitude with delayed timing (Figure 3B, blue tracings) are compared to the normal response (black tracings). These morphologically distinct pathological ERGs are indistinctively interpreted as either low voltage with normal timing (Class #1) or low voltage with delayed timing (Class #2). Modified from Dr. Pierre Lachapelle's teaching slides (with permission).

As it can be seen, in either of the two diagnostic categories, the b-waves present with very different morphologies. Nonetheless, the pathological ERGs shown at Figures 3A-B are interpreted as either low-voltage with normal timing (Class #1) or as low-voltage with delayed timing (Class #2). Therefore, within each of the two diagnostic classes, the ERGs are indistinguishable. The question is, should ERGs of such strikingly different morphologies be classified as equivalent ERGs? The answer to the latter question is most probably "no". Indeed, if the contribution of each ERG component (i.e. a-wave, b-wave, oscillatory potentials, etc.) was always equally altered by any retinal dystrophy, disease, or anomaly, the ERG waveform could change in amplitude, but would always preserve its normal waveform morphology given that the relative weight of each ERG component

would always bring the same contribution to the ERG waveform. However, pathological ERG waveforms in Figures 3A and 3B (blue and green tracings) suggest that this is not always the case given that these ERG waves have, upon visual inspection, extremely different morphologies and complexity (i.e. number of clearly identifiable components). Likewise, through the photopic hill function, the change in ERG amplitude is also accompanied by variations in the overall ERG waveform morphology (Garon et al., 2014). Similar to what was shown with the above pathological ERG examples (Figures 3), the traditional analysis of the normal photopic hill reveals b-waves that have similar amplitude and peak time despite presenting with strikingly different morphologies. Therefore, certain normal ERGs are also indistinguishable based on their traditional analyses and further illustrate the limitations of the current TD parameters. Of course, qualitative description of the ERG waveform morphology can be used to categorize ERGs, the best example being the pathognomonic "square-wave-like a-wave" and "truncated b-wave" morphology known to characterize the photopic ERG of patients affected with CSNB (Heckenlively et al., 1983; Lachapelle et al., 1983; Miyake et al., 1997; McAnany et al., 2013). However, while profound morphological alterations of the ERG (such as in CSNB) are easy to identify on visual inspection, one cannot exclude (when looking at Figures 3A, 3B) the possibility that pathologies could modify, to different extent and in countless ways, the morphology of ERGs recorded from patients affected with other retinal disorders.

3.5.2 Quantification of ON and OFF responses

As aforementioned, a long-flash (e.g. > 150 ms) can be used to allow the separation and a more selective evaluation of the retinal ON (b-wave) versus OFF (d-wave) pathway (Sieving et al., 1994, Ueno et al. 2006, Miyake et al., 1987; Alexander et al., 1992; Sieving 1993; Ruether and Kellner 1998). However, long-flash ERG protocols are not built-in in all ERG systems, potentially because this test is not yet part of the ISCEV standards for full-field electrophysiology. Nonetheless, long-flashes can be performed as an additional test with some ERG systems, but is it not commonly used in clinical electrophysiology and often require more cooperation from the subjects/patients. Furthermore, the amplitudes of the b- and d-waves thus obtained are significantly smaller than the regular short-flash ERG waveform, which might complicate the analyses of severely attenuated responses. As a possible remedy to the above limitations of the long-flash ERG, it was recently shown that fitting the photopic hill (evoked to short-flashes) with the Glasgow equation, which combines a Gaussian and a logistic growth function (Hamilton et al., 2007), could be used to study the ON (i.e. logistic component) and OFF (i.e. Gaussian component) retinal pathway, respectively (Hamilton et al., 2007, Garon et al., 2014). However, the complete process may undesirably take additional clinical time. Indeed, to record the photopic hill and derive an optimal Glasgow fit, many stimulus intensities must be used. Furthermore, each of the photopic hill responses is often obtained from the average of several flashes (10-100 trials). Similarly, the analyses are also long given that we need to measure the ERG parameters of all responses, and generally in both eyes. Following the quantifications, the mathematical model (Glasgow equation) must be optimally fitted to the b-wave amplitude values and interpreted. In addition to the limitations of these time-consuming steps is the problem of ill-defined best-fit solutions. For example, the same photopic hill can be fitted using various combinations of the Logistic and Gaussian components resulting in almost identical Glasgow curves. When taken together with the time-consuming recordings, this ON-OFF quantification approach seems suboptimal. In summary, the above points demonstrate the current limitations of ON and OFF measurements. Optimally, we should be able to decompose the ON and OFF components of the standard photopic ERG without the use of long-flashes or curve fitting of the photopic hill.

3.5.3 Quantification of severely attenuated ERGs

Additionally, at the end-stage of severe degenerative retinopathies (such as RP), nearly-extinguished photopic ERGs are usually the last measurable signs of retinal function (Berson, 1993; Rispoli et al., 1994). Since the end-stage of degenerative retinopathies represents a good time-window to perform clinical trials of new drugs, it is as important to quantify the response at the end-stage of the disease as it is earlier. It is also imperative because patients sometimes present with residual ERGs at their first visit at the clinic when the diagnostics are not necessarily known. However, residual photopic ERGs are difficult to analyse (and thus categorize) in the TD, given their low-amplitudes, low signal-to-noise ratios (SNR), and minimal morphological features reminiscent of the normal ERG response. Of course, the lower the SNR, the more obtrusive the background noise and

consequently, the more difficult is the identification of the ERG components. In these conditions, accurately extracting relevant information from the time domain becomes harder, if not impossible. Indeed, it is well documented that the background signal noise can compromise the accurate measurement of the amplitude and/or peak time of the different ERG components, consequently altering their interpretation. Clearly, an analytical approach that could help identify and more accurately quantify reproducible features of the ERG in residual or noisy responses is needed. The averaging of several responses represents the traditional approach to increase the SNR, but this technique can be time-consuming when several trials need to be averaged (i.e. which is habitually the case for end-stage clinical ERG recordings). Other alternatives have been suggested to facilitate the recording and clinical interpretation of nearly extinguished ERGs. These methods either necessitate the use of specially designed stimuli, such as high flickering rate of presentation and/or specific analog filtering approaches to optimise the extraction of the remaining ERG signal from the increasingly overwhelming noise (Birch and Sandberg, 1996; Andreasson, 1988). While the above techniques improve the recording of residual ERGs, the resulting waveforms, when present, often have little in common with what we know of the normal ERG signal, thus preventing comparisons with previous recordings obtained from the same subject.

4. ADVANCED ANALYSIS APPROACHES

Today, the research/clinical potential of powerful mathematical tools specifically designed for signal analysis have reached new levels, and biopotentials can now be quantified way beyond their amplitudes and/or peak times (Van Drongelen, 2007). As we will see below, it is thus surprising that little has been done so far in applying more advanced signal processing approaches to basic ERG research to improve its clinical applications. Based on the above, this PhD project aimed at extensively applying state-of-the-art analysis approaches to the ERG to overcome the above limitations of the TD measurements and improve the usefulness and our understanding of the photopic ERG signal in both basic research and clinical applications. Below, advanced methods that were explored throughout this thesis are presented and reviewed.

4.1 The frequency domain

Even before the discovery of the ERG, Jean Baptiste Joseph Fourier proved (in 1822) that any periodic function could be represented as an infinite sum of weighted sines and cosines, a concept that was later referred to as the *Fourier series*. From this formalism emerged the *Fourier transform* and, eventually, very fast algorithms, such as the fast Fourier transform (FFT; Cooley and Tukey, 1965), were developed to fasten the computation process. The Fourier transform decomposes a signal (i.e. a time series) into the frequency components that build up that signal. As a result, the Fourier transform of a time series (i.e. time domain) is a function of frequency (i.e. frequency domain), and its values provide the weight (i.e. the importance) that each frequency contributes to the original time domain signal (Van Drongelen, 2007). Nowadays, Fourier analysis remains a very useful and widely used approach for extracting frequency information from biopotentials.

4.1.1 Applications to the ERG

Since the end of the sixties, frequency domain analyses, such as the FFT, have been relatively frequently applied to the analysis of the ERG (Poppele and Maffei, 1967; Breslin and Parker, 1973; Gur and Zeevi, 1980; Van der Torren et al., 1988; Sieving et al., 1998; Wood et al., 2014; McAnany and Nolan, 2014). However, as aforementioned, Fourier analyses were initially developed for periodic functions (i.e. functions that continuously cycle in time) such as square-waves, triangular-waves, sinusoidal-waves and the like, and/or any combination of any periodic functions (Mallat, 2009; Van Drongelen, 2007). On the other hand, in the present work, we want to characterize fluctuations (e.g. a-wave or b-wave) that are localized in time (transient). Nevertheless, in the Fourier domain, a local oscillation will always be ill-defined, as local oscillations always recruit several modes in Fourier, which better deals with stationary oscillations.

Despite being suboptimal for non-periodic responses, the FFT can nonetheless be applied to any non-periodic signal over a time interval. As such, frequency domain analyses were previously conducted on the single-flash ERG and revealed that the response was composed of several frequency components, thus unveiling the composite nature of the full-field ERG signal (Gur and Zeevi, 1980; Sieving et al., 1998). Studies reported that the single-flash ERG was composed of a large low-frequency (between 15-50 Hz) component reflecting the slow waves (i.e. a- and b-waves) as well as smaller components of high-frequency (between 75-200 Hz) accounting for the OPs (Poppele and Maffei, 1967; Breslin and Parker, 1973; Gur and Zeevi, 1980). Intuitively, the morphology of a given signal is most probably determined by how its different frequency components (i.e. the ingredients of the ERG) combine to make the waveform, and FFT spectra that would not show the same frequency components could hence potentially highlight morphological differences between ERG waveforms. Surprisingly, however, the FFT was never used as a means to investigate the ERG morphology. Rather, it was used to measure the magnitude (or power) of the different frequency components that contribute to the ERG signal. Of interest, in 1980, Gur and Zeevi successfully demonstrated that the FFT features of the ERG were of much lower variability than that of the TD descriptors, and showed that the FFT descriptors could be extracted even from noisy recordings (Gur and Zeevi, 1980). More details regarding the computation of the FFT are provided in Appendix I (Chapter IX).

4.2 <u>The time-frequency domain</u>

As indicated above, Fourier transforms enable the decomposition of a signal into its overall frequency components using a collection of weighted periodic trigonometric functions of different frequencies. While Fourier analyses are well-suited to identify and estimate the magnitude of the main frequency components of a periodic signal, they should be interpreted with great care when analysing non-periodic signals such as the ERG. Indeed, Fourier methods perform well in identifying the major frequency components of the ERG response, but they cannot be used to determine the time at which they occur in the signal. This lack of temporal resolution results in frequency components might be overestimated, and/or underestimated, and very local transient features can be obscured. To overcome these drawbacks, an approach that combines both time and frequency information is warranted.

As a first solution, Gabor (1946) proposed to use a *Short-Time* Fourier Transform (STFT) in order to analyse small sections of the signal at a time by windowing the area of

interest. While the STFT can add some temporal resolution to the FFT, it often results in an inadequate temporal or spectral resolution, depending on the selected analysis window's length (which needs to be adjusted on a case by case basis to avoid those resolution problems). In order words, the STFT cannot simultaneously capture both short-durationhigh-frequency, and long-duration-low-frequency information.

Of interest, the above limitations of the FFT and STFT can be overcome with the use of a relatively recently developed family of signal processing techniques known as the wavelet transform (WT). The WT has the advantage of optimally combining both time and frequency information into a single two-dimensional representation (often called a scalogram), which allows the detection of transient oscillations occurring at different times and/or frequencies (Van Drongelen, 2007; Mallat 2009). In a time-frequency scalogram, the representation of local a oscillation is always much sparser than with a Fourier transform, and this is the great idea of the wavelet transform: finding the most efficient representation for a signal of interest. In short, the fact that the a- and b-waves are local fluctuations completely justifies their quantification in the time-frequency plane, where we should be able to use few modes to optimally represent them. A Fourier decomposition will never give this kind of information for local oscillations.

Another great advantage of the wavelet transform is the hundreds of mother wavelet function types that can be chosen to more suitably describe and analyse the features of an investigated signal (Van Drongelen, 2007; Mallat, 2009). The latter clearly contrast with Fourier Analyses which are strictly limited to sinusoidal functions. Given the above advantages, the WTs have become the most-widely used, and favored time-frequency analysis tools to analyse signals and images across a widespread umbrella of scientific areas (e.g. medicine, engineering, physics, etc.), and keep on gaining in popularity in their applications to the analysis of various biomedical signals/images (Addison, 2002; 2005). While modern wavelet analyses date from the mid-1980s (Morlet and Grossmann, 1984), they were, at first, almost entirely restricted within a small community of mathematicians, until the nineties, where interest exploded in all fields of science and engineering. There was then a very rapid growth in the number of investigators converting their FFT applications with wavelets, and the last few years have each seen the publication of over 1000 peer-reviewed journal papers which cover nearly all fields of science. In medicine, when compiling the results of a quick abstract/title search for *wavelet transform* on PubMed, the first instance of a medical wavelet study is in 1988, and since 1994, the rate of publication has been increasing exponentially to reach close to 3000 cumulative publications in 2016. When reviewing this scientific literature, one encounters widespread applications for the WT dedicated to the analysis of complex physiological signals such as the EEG, ECG, and EMG, to name a few (Constable and Thornhill, 1993; Demiralp and Ademoglu, 2001; Jia et al., 2006). However, only 19 studies to date (excluding ours) have applied WTs to the ERG signal (i.e. Pattern ERG, Flash ERG, multi-focal ERG, etc.) (Barraco et al., 2007, 2010, 2011a, 2011b, 2014; Dimopoulos et al., 2014; Forte et al., 2008; Kundra et al., 2006; Miguel-Jiménez et al., 2008, 2010, 2011, 2015; Nair and Joseph, 2014; Penkala 2005, 2007, 2010; Rogala et al., 2005; Varadharajan et al., 2000, 2007). These studies will be briefly reviewed in the following sections. Two main types of wavelet analysis will be presented and reviewed: the continuous (section 4.2.1) and the discrete (section 4.2.2) types.

4.2.1 The continuous wavelet transform (CWT)

The continuous wavelet transform is a relatively recent alternative to Fourier analyses (FFT and STFT) that was first introduced in the eighties by Morlet and Grossmann (1984). The CWT is obtained by iteratively correlating the signal to scaled and translated version of a small oscillating function called the mother wavelet (MW) (Van Drongelen, 2007; Mallat, 2009). Each correlation [i.e. computed for every scale (frequency) and translation (time) combinations of the wavelet] leads a coefficient quantifying the match found between the wavelet and the considered signal. These so-called *wavelet coefficients* can then be plotted to provide a time-frequency representation of the fluctuations in the signal. More details regarding the computation of the CWT are provided in Appendix II (Chapter IX).

4.2.1.1 Advantages of the CWT

The main advantage of the CWT is that it precisely combines time and frequency information in a single time-frequency representation, thus allowing the detection of transient oscillations/components occurring at different times or different frequencies in the signal (Van Drongelen, 2007; Mallat, 2009). Differently from the FFT, which presumes that the signal is periodic, the CWT can analyse the spectral content of a signal at any given time using an optimal time-frequency grid. The CWT provides excellent temporal resolution and poor spectral resolution at high frequencies and good frequency resolution and poor time resolution at low frequencies. The latter is optimal as most biological signals have low-frequency components that spread over long durations and high-frequency components that spread over short durations (Van Drongelen, 2007).

4.2.1.2 Applications to the ERG

As summarized in Table 1, CWT techniques ([using different types of MW) were occasionally applied to the analysis multifocal ERG (mfERG), of pattern ERG (PERG), and full-field ERGs, with only six first-authors contributing to a mere 12 research articles (Barraco et al., 2007, 2010, 2011a, 2011b, 2014; Dimopoulos et al., 2014; Forte et al., 2008; Miguel-Jiménez et al., 2015; Nair and Joseph, 2014; Penkala, 2005, 2007, 2010), in which only two studies included photopic ERG analyses (Dimopoulos et al., 2014; Nair and Joseph, 2014).

1st Author	Year	Electroretinographic Signal	Mother Wavelet (CWT)	Analysed components of the full-field ERG
Miguel-Jiménez	2015	multifocal ERG	Morlet	N/A
Nair	2014	Scotopic and Photopic ERG, Flicker	Mexican Hat, Morlet	N/A
Dimopoulos	2014	Scotopic and Photopic ERG*	Morlet	OPs
Forte	2008	Rodent Scotopic and Photopic ERG*	Morlet	OPs
Barraco	2014	Scotopic ERG	Mexican Hat	a-wave
Barraco	2011b	Scotopic ERG	Mexican Hat	a-wave
Barraco	2011a	Scotopic ERG	Mexican Hat	a-wave
Barraco	2010	Scotopic ERG	Mexican Hat	a-wave
Barraco	2007	Scotopic ERG	Mexican Hat	a-wave
Penkala	2010	Pattern ERG	Coiflet and Morlet	N/A
Penkala	2007	Pattern ERG	Mexican Hat, Morlet	N/A
Penkala	2005	Pattern ERG	Mexican Hat	N/A

Table 1. Compilation of all CWT studies ever applied, to our knowledge, on any type of electroretinographic responses as of October 2016. Years in bold indicate the first year at which each author first contributed. The stars (*) indicate the only two studies that included some photopic ERG analyses.

As reported in Table 1, Penkala (2005) was the first to apply the CWT to an ERG waveform. His work focused on the analysis of Pattern ERG waveforms using the Mexican Hat (Penkala, 2005), Morlet (Penkala, 2007, 2010) and Coiflet MW (Penkala 2010). In his latest study, Penkala used a Morlet CWT method to compare pattern ERG responses obtained from normal subjects and patients affected by glaucoma (Penkala 2010). This study, along with his previous reports (Penkala, 2005, 2007) showed that the variability of CWT-derived pattern ERG descriptors was smaller than the traditional TD-based descriptors, thus permitting the earlier detection of significant pathological changes (that were unnoticed using TD measures) thus improving the separation between normal and abnormal PERG waveforms (Penkala, 2010). Barraco et al. (2007) was the second to apply the CWT to an electroretinographic waveform. His work was dedicated to the analysis of the scotopic a-wave using the Mexican Hat wavelet (Barraco et al., 2007, 2010, 2011a, 2011b, 2014). Barraco et al.'s studies revealed that the scotopic a-wave of normal subjects was composed of three main frequency components, namely 20, 140 and 180 Hz (Barraco et al., 2007; 2011b). They also showed a predominance of the low-frequency components in the a-wave and found that their time distribution depended more on the luminance compared to higher frequency components of the a-wave (which are less affected by the luminance) (Barraco et al., 2007). He also used the CWT to compare the scotopic a-wave of normal subjects with CSNB patients (Barraco et al., 2010, 2014) and achromats (Barraco et al., 2010, 2011a, 2014). Of interest, the higher frequency component was absent in achromats, and the frequency of the low-frequency component was dramatically reduced in CSNB patients, suggesting that the CWT could also help in the diagnosis of retinal diseases (Barraco et al., 2010, 2011a, 2014). Finally, in their latest study (Barraco et al. 2014), they compared the efficacy of various techniques (Fourier analysis, principal component analysis, and wavelet analysis) in separating pathological scotopic a-waves from the healthy ones. Their latest findings demonstrated that both principal component and Fourier analysis of scotopic ERGs did not add useful clinical information for the diagnosis of retinopathies, whereas the use of the CWT provided a more powerful analysis tool (Barraco et al., 2014). In 2008, Forte et al. used the CWT analysis (using the Morlet MW) and revealed that, in rodents, two frequency components (i.e. 70-80 Hz and 120-130 Hz) contributed to the genesis of the OPs (Forte et al., 2008). This study also showed that

frequency, the latency, and the energy level of the two OP frequency bands differently varied with stimulus intensity, suggesting that they might be evoked by different retinal elements/mechanisms (Forte et al., 2008). Following the work of Forte et al. (2008), Dimopoulos et al. (2014) studied the OPs of normal human subjects with the Morlet MW. They revealed that the scotopic OPs only had one main oscillatory high-frequency band at 150-155 Hz, which was unaffected by age. In contrast, photopic OPs had two different bands: a low-frequency band oscillating at about 70-80 Hz (time-locked to late OPs) and a high-frequency band at about 130-150 Hz (time-locked to early OPs) (Dimopoulos et al., 2014). They also revealed that by 60 years of age, there was a consistent energy reduction specific to the low-frequency OP band. They suggested that these two distinct bands could possibility represent the ON vs. OFF system (Dimopoulos et al., 2014). Nair and Joseph (2014) similarly applied the CWT to full-field and flicker ERGs using the Mexican hat and Morlet MW with the aim of automating ERG analyses. They applied their method to normal subjects as well as patients affected with CSNB, rod-cone dystrophies, and CRVO, and found significant differences between the maximal wavelet coefficients of control vs. patients (Nair and Joseph, 2014). They concluded that the CWT analysis was reliable enough to build an automated diagnostic platform (Nair and Joseph, 2014). Finally, more recently, Miguel-Jiménez et al. (2015) investigated the application of the CWT analysis (using the Morlet MW) of mfERG responses in the diagnosis of glaucoma. The maximal wavelet coefficients were used as inputs to a neural network, and the authors obtained a classification sensitivity and specificity of 0.894 and 0.844, respectively (Miguel-Jiménez et al., 2015). The latter demonstrate a more reliable detection of normal and glaucomatous sectors with the CWT compared to the TD. In summary, the above studies showed that the analysis of normal and pathological ERGs with the CWT can potentially reveal subtle (and possibly diagnostic) changes that are almost impossible to appreciate with TD measures.

4.2.2 The discrete wavelet transform (DWT)

By the end of the nineteen eighties, major advances in wavelet theory were made possible due to the development and better understanding of families of compact mother wavelet that had *orthonormal bases* (Meyer, 1987; Daubechies, 1988). With such wavelet families, wavelet transforms that would require only a small amount of processing without any loss of information were made possible (Mallat, 1989a). This promising type of highly efficient wavelet transform was termed *discrete wavelet transform* or DWT. In contrast to the CWT, which operates over every possible scale and translation values, the DWT uses a very specific subset of optimal scales and translations. As a result, the DWT does not process any redundant information, thus saving a significant amount of computation time and resources (Mallat, 2009). The latter breakthrough permitted the advance of the wavelet theory to the next level and made wavelet transformation particularly well suited for the realm of digital computation, which was also booming at that time (Daubechies, 1990). Since then, the DWT applications have been constantly growing and the DWT has become the most widely used wavelet method for signal processing applications. It is hard to realize to what extent, but nowadays, the DWT is literally everywhere. Virtually all digital images that you have ever looked at on a web browser were compressed using discrete wavelets (Mallat, 2009). Similarly, to transport a large amount of video data through a data cable, the information is often compressed, sometimes using less than 10% of the initial memory, but still displaying more than 90% of the image quality. Of interest, with this new type of efficient discrete wavelet transforms was put forward the notion of *multiresolution* analyses, first developed by Stéphane Mallat and Yves Meyer (Meyer, 1987; Mallat, 1989a, Mallat, 1989b). A multiresolution analysis decomposes the signal into a subset of signals with different resolutions. The latter allows the analysis and synthesis of a signal from its coarser components to the most detailed view of its finest features (Mallat, 2009). Using this multiresolution decomposition property of the DWT, we should be able to optimally decompose, analyse, denoise and reconstruct the ERG signal. Details regarding the computation of the DWT are provided in this thesis in Appendix III (Chapter IX).

4.2.2.1 Advantages of the DWT

According to Heisenberg's uncertainty principle, it is absolutely impossible to know the exact frequency and exact position of an oscillatory component in the same representation (Burrus et al., 1998). Indeed, Fourier analysis can compute the exact spectral content that exists in a signal, but cannot give any information about the temporal distribution of each component. On the other hand, time-amplitude analysis can reveal the exact time location of an oscillation, but without showing any quantitative information

about its frequency. In other words, an oscillation within a signal cannot simply be represented as a point in the time-frequency space (such as with the CWT); rather it must be represented as a "rectangular box". Of interest, this is exactly what the DWT scalogram exhibits; a time-frequency (T-F) representation of the signal (i.e. scalogram) that is broken down into boxes of various dimensions, each associated to a unique wavelet coefficient and each exhibiting an optimal compromise between time and frequency resolution (i.e. accuracy). Of interest, high frequencies are associated with the signal over a fine time scale allowing information about the timing of fast oscillation (e.g. the OPs) to be preserved at the cost of a poorer spectral resolution (the exact frequency is uncertain). Oppositely, the low frequencies (e.g. a- and b-waves) are associated with coarse time scale allowing the information about the frequency to be well-preserved at the expense of an imprecise temporal resolution (the exact timing of the oscillation is uncertain). This is why DWT analyses are often referred to as multiresolution analyses. The detection of small bandwidth changes necessitates a high spectral resolution and the detection of larger bandwidth changes necessitate less frequency resolution. This characteristic of the DWT will be very practical in our study since the a- and b-waves' bandwidth (20-40Hz; i.e. a bandwidth of 20 Hz) is smaller than that of the OPs (75-200Hz; i.e. a bandwidth of 125 Hz) and thus the DWT should be an optimal ERG analysis tool by providing the necessary high spectral resolution for the a- and b-waves, and less spectral resolution for the OPs (which does not need as much spectral resolution given that they span a large bandwidth). Moreover, the DWT is exempt of redundancy and thus necessitates far less computation time than any other wavelet transforms (Mallat, 2009). The non-redundancy, also allows the inverse DWT (IDWT; i.e. the inverse operation that allows to pass from the DWT scalogram back to the signal) to perfectly reconstruct (signal synthesis) the signal (without any error). Of interest, the IDWT can also be used to de-noise the signal. Briefly, corrections can be carried out on the wavelet coefficients by setting all wavelet coefficients that have a value smaller than a pre-defined value to zero. The remaining non-zero wavelet coefficients are then used reconstruct a compressed copy of the original signal (i.e. the IDWT) by using only pertinent information (Quiroga and Garcia, 2003). As a result, the reconstructed signal is commonly far less noisy (higher SNR) (Quiroga and Garcia, 2003). In summary, given the above advantages, the DWT should be an appropriate tool to decompose and analyse the ERG signal in the time-frequency domain. Like a mathematical magnifier, the multiresolution organization of the DWT should allow us to optimally pinpoint local ERG signal behaviors (a-wave, b-wave or OPs), to analyse signal features (e.g. complexity) or "zoom out" to get a global Fourier-like view of the signal (e.g. when averaging at the global energy on each band). The unique presentation of the DWT scalogram should also facilitate the identification of relevant DWT coefficients/descriptors of the ERG waveform, which are needed to optimally quantify the morphology of the ERG signal. Additionally, one should more easily detect and reconstruct residual ERG responses buried in low SNR recordings.

4.2.2.2 Applications to the ERG

As aforementioned, to date, few attempts have been made to evaluate the usefulness of the wavelet techniques, such as the CWT, to the field of electroretinography. Despite the unique advantages of the DWT, even fewer attempts have been made to evaluate the usefulness of the DWT to the analysis of the ERG waveform. As summarized in Table 2, DWT techniques (using different types of MW) were sporadically applied to the analysis of the pattern ERG, multifocal ERG, flicker ERG and full-field ERG, with only 8 (excluding ours) published research articles (Miguel-Jiménez et al., 2008, 2010, 2011; Nair and Joseph, 2014; Rogala et al., 2005; Varadharajan et al., 2004; Kundra et al., 2016).

1st Author	Year	Signal	Mother Wavelet (DWT)	Analysed components of the full-field ERG
Kundra	2016	Photopic ERG	Daubechies	PhNR
Nair	2014	Scotopic and Photopic ERG, Flicker*	Haar	N/A
Miguel-Jiménez	2011	multifocal ERG	Bior3.1	N/A
Miguel-Jiménez	2010	multifocal ERG	Bior3.1	N/A
Miguel-Jiménez	2008	multifocal ERG	Bior3.1	N/A
Rogala	2005	Pattern ERG	Daubechies	N/A
Varadharajan	2007	Scotopic ERG	Daubechies	a-wave, b-wave, OPs
Varadharajan	2000	Scotopic ERG	Daubechies	N/A

Table 2. Compilation of all DWT studies ever applied on any type of electroretinographic responses as of October 2016. Four first authors contributed to a total of 8 research articles. Years in bold indicate the first year ay which each author first contributed. The star (*) indicate the only study that included some photopic ERG analyses.

As reported in Table 2, Varadharajan and colleagues (2000) were the first authors to apply the DWT to any kind of ERG responses. Their work focused on the analysis of the full-field scotopic ERG waveform using the Daubechies (D4) mother wavelet (Varadharajan et al., 2000, 2007). In their first study, Varadharajan and colleagues (2000) used Mallat's multiresolution decomposition to display the time domain reconstruction of the wavelet coefficients obtained at seven frequency bands (i.e. using the IDWT) in normal subjects and patients affected with Duchenne muscular dystrophy (Varadharajan et al., 2000). Using this technique, they found significant differences between controls and patients, at all frequency bands. They also concluded that the reconstructed DWT coefficients, obtained at different frequency bands, were independent of each other and could potentially represent independent physiological processes. Similarly, in 2007, Varadharajan and colleagues (2007) went further by showing that it was also possible to specifically approximate the TD a-wave, b-wave, and at least one OP using the inverse DWT of the scotopic ERG waveform by reconstructing specific wavelet coefficients (Varadharajan et al., 2007). Next, Rogala and Brykalski (2005) were the second authors to apply the DWT to an electroretinographic waveform. In their DWT study, they used the Daubechies mother wavelet to compare pattern ERG responses obtained from normal subjects and patients. When used as input variables to a principal component analysis (or PCA; i.e. a statistical technique used to segregate data), the DWT features were shown to be superior to traditional TD measures in separating normal and pathological PERG waveforms (Rogala and Brykalski, 2005). Furthermore, the misclassification rate of traditional time domain parameters ranged between 55-60% while that obtained with the DWT was of 34-36% (Rogala and Brykalski, 2005). Hereafter, Miguel-Jiménez and colleagues (2008) were the third group to apply the DWT to an electroretinographic signal. Their work on DWT research so far includes 3 research papers, each limited to the analysis of the global-flash multifocal ERG waveform using the Bior3.3 mother wavelet (Miguel-Jiménez et al., 2008, 2010, 2011). In their latest study, Miguel-Jiménez and colleagues used the DWT method to compare multifocal ERG sectors obtained from normal subjects and patients affected with glaucoma (Miguel-Jiménez et al., 2011). This study, along with their previous reports (Miguel-Jiménez et al., 2008, 2010) showed that this approach could potentially be more reliable to detect changes in glaucoma patients, compared to traditional Humphrey visual field tests. Likewise, a more sensitive detection of normal vs glaucomatous sectors was obtained with the DWT compared to the TD (Miguel-Jiménez et al., 2011). Fourthly, Nair and Joseph (2014) applied the DWT to full-field and flicker ERGs using the Haar wavelet. They were first to apply the DWT to the full-field photopic ERG response. They limited their DWT analyses to 3 level of decomposition and extracted the maximal wavelet coefficient from the full-field scotopic and photopic ERGs as well as flicker ERG. They applied this decomposition to normal subjects as well as patients affected with CSNB and cone-rod dystrophies, and found significant differences between the maximal approximation coefficient of control and patients (Nair and Joseph, 2014). In summary, the above studies demonstrated that the analysis of normal and pathological ERGs with the DWT exposes subtle changes that are more sensitive than TD measures, thus leading to significant diagnostic improvements. The application of the DWT to the photopic ERG remains however very limited, and the current PhD thesis is indented to help address this gap.

5. THESIS SPECIFIC OBJECTIVES

In summary, the ERG is a composite signal produced by the retina following a flash-like stimulus, and specific components of the signal (such as a-wave, b-wave and OPs) have been suggested to arise from different retinal structures/pathways. To date, the ERG remains the most objective tool to noninvasively assess retinal function, and continues to be widely used to confirm the diagnosis and monitor the progression of a variety of retinal disorders. It is also extensively used to study the underlying retinal physiology (basic science). Despite numerous advancements in ERG technologies and knowledge, the analysis of the ERG remains mostly limited to time domain (TD) measurements (amplitude and timing) of the a- and b-waves. This practice has been proven and standardized (McCulloch et al., 2015) but, as suggested above, it might nonetheless severely limit the interpretation of the ERG signal. In summary, the traditional analysis of the photopic ERG in the time domain has three main limitations:

L1: The amplitude and peak time measurements of the a- and b-waves are inadequate to quantitatively describe all photopic ERG features (such as its morphology, complexity, frequency, etc.), and therefore limit the number of normal and pathological classes that one can identify.

L2: The amplitude and peak time measurements of the a- and b-waves are not able to dissociate the ON and OFF components of the photopic ERG unless using a particular long-flash stimulus or a significantly longer protocol (recording of luminance series).

L3: When the responses are partially or entirely buried in noise, the accuracy of amplitude and peak time measurements of the photopic ERG becomes compromised, and in the worst case, the measurements are impossible.

In summary, the practice of interpreting the ERG solely in the time domain seriously impairs:

1-The capacity to detect an anomaly at disease onset and/or the ability to detect small changes between ERGs, etc.

2-The capacity to distinguish between different ERGs or components and the ability to identify the retinopathy the patient is suffering-from.

Given that any of the photopic ERG components can theoretically be the first to be affected by a given disease process, it is imperative to quantify the specific contribution of each of these components to the genesis of the signal. The latter could allow us to detect signs of retinal malfunction more reproducibly and at the earliest stage possible (sensitivity) so that intervention (when available) can yield optimal results. Likewise, in the above introduction, several examples illustrated the need for new tools and novel approaches that could help us reveal reproducible descriptors that can discriminate between ERGs (specificity) that are, to this date, erroneously considered as equivalent. Advanced signal processing techniques, such as frequency domain analysis, and more recently, wavelet transform analysis, allowed the extraction of additional useful information in signals such as the electroencephalogram (EEG) or the electrocardiogram (ECG), thus significantly improving the sensitivity and specificity of the analysis. Therefore, adapting this technology to the ERG field could potentially improve the usefulness of the ERG in basic research, but more importantly in its clinical applications. However so far, very few attempts have been made to evaluate the usefulness of advanced techniques, such as the CWT and DWT, to the field of electroretinography. Indeed, the novelty of this research field certainly opens the door to new findings and motivated me to explore this essentially under-exploited research field (e.g. DWT analyses of the ERG). Given the above, the main goal of this doctoral project was to analyse the human photopic ERG signal using state-ofthe-art signal processing techniques to answer the following question: "*can advanced analytical approaches uncover additional useful information in ERG recordings*?" More specifically, we address this question using four specific aims (SA). Can we use advanced approaches to:

SA1: Better distinguish between photopic ERGs presenting with diverse morphologies, therefore refining normal and pathological ERGs classification (specificity)?

SA2: More specifically quantify the ON and OFF responses of the photopic ERG (specificity)?

SA3: Facilitate the detection of imminent retinal dystrophies at their earliest stage possible (sensitivity)?

SA4: Facilitate follow-up of disease progression in patients initially presenting with a nearly extinguished ERG and enable the analysis of noisy ERGs in general (sensitivity)?

The above questions/aims of this project were covered by a total of five manuscripts which are all included below (see Chapters II to VI). The findings presented in this thesis could trigger a new ERG research area and open the discussion to improve the standards of care and analyses in the field of visual electrophysiology.

1. Preface to Chapter II.

As aforementioned, in the last 150 years or so, the ERG went through several breakthroughs in recording technologies (e.g. amplifiers, digital filters, personal computers and softwares) and recording electrodes (i.e. less invasive and more efficient electrodes). Despite these advancements in ERG technologies and the enhanced quality of ERG signals thus obtained, the ERG analysis is still mostly limited to amplitude and peak time measurements of its two major components (i.e. the a- and b-waves). In fact, even the latest (2015) photopic ERG standard of the International Society for Clinical Electrophysiology (ISCEV) recommends to use the 3.0 cd.s.m⁻² stimulus intensity and to analyse the a- and b-waves amplitude and peak-time (i.e. analyses in the time domain). This practice has been proven but, as shown in the above thesis introduction, it limits the interpretation of low voltage ERGs, restricts the number of relevant ERG descriptors and does not consider the morphology of the signal. Advanced signal processing techniques, such as frequency domain analyses, or more recently, time-frequency domain analyses, allowed extraction of additional useful information in signals as the electroencephalogram (EEG) or electrocardiogram (ECG), thus significantly improving their diagnostic potential. Nevertheless, these techniques have only been sporadically applied to the electroretinogram (ERG). To investigate whether more advanced approaches could be instrumental in extracting more useful information from the ERG, we compared the traditional time domain (TD) approach with analyses conducted with the fast Fourier transform (FFT), the continuous wavelet transform (CWT) and the discrete wavelet transform (DWT). Determining which advanced technique was better suited for ERG analysis was the purpose of this first study. Each method extracted relevant information from the signal but had distinct limitations (e.g. temporal or frequency resolution). The DWT offered the optimal compromise by allowing us to extract additional relevant descriptors of the photopic ERG signal at the cost of lesser temporal or frequency resolution. Follow-ups of disease progression were also more prolonged with the DWT. This first study allowed us to conclude that the DWT method should allow more sensitive/specific quantifications of ERG responses and facilitate follow-up of disease progression.

2. Manuscript Title.

CHAPTER II

Manuscript 1 (Published Original Research Article)

ADVANCE IN ERG ANALYSIS: FROM PEAK TIME AND AMPLITUDE TO FREQUENCY, POWER AND ENERGY

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3. Abstract

Purpose. To compare time domain (TD: peak time and amplitude) analysis of the human photopic electroretinogram (ERG) with measures obtained in the frequency domain (Fourier analysis: FA) and in the time-frequency domain (continuous (CWT) and discrete (DWT) wavelet transforms). Methods. Normal ERGs (n=40) were analyzed using traditional peak time and amplitude measurements of the a- and b-waves in the TD and descriptors extracted from FA, CWT, and DWT. Selected descriptors were also compared in their ability to monitor the long-term consequences of disease process. Results. Each method extracted relevant information but had distinct limitations (i.e., temporal and frequency resolutions). The DWT offered the best compromise by allowing us to extract more relevant descriptors of the ERG signal at the cost of lesser temporal and frequency resolutions. Follow-ups of disease progression were more prolonged with the DWT (max 29 years compared to 13 with TD). Conclusions. Standardized time domain analysis of retinal function should be complemented with advanced DWT descriptors of the ERG. This method should allow more sensitive/specific quantifications of ERG responses, facilitate follow-up of disease progression, and identify diagnostically significant changes of ERG waveforms that are not resolved when the analysis is only limited to time domain measurements.

4. Introduction

The electroretinogram (ERG) identifies the electrical signal that is generated by the retina in response to a light stimulus. It is the first biopotential ever recorded from a human subject, namely, by Dewar in 1877 [1]. However, despite significant (r)evolution in the recording technologies (essentially from the string galvanometer to the digital amplifier and supporting computer software) and, consequently, the significantly enhanced quality of the ERG signal thus obtained, analysis of the ERG remains for the most part limited to amplitude and peak time measurements of its major components, namely, the a- and bwaves. This is at least what is recommended in the ERG standard of the International Society for Clinical Electrophysiology of Vision (ISCEV) [2]. The a- and b-waves of the ERG are said to reflect the activity generated by the photoreceptors and the Bipolar-Müller cell complex, respectively [3–5]. These components are usually referred to as the slow waves of the ERG. Also identified in the ERG signal are the small, high-frequency, oscillations that are often seen riding on the ascending limb of the b-wave [6, 7]. These components, referred to as oscillatory potentials (OPs), are most probably generated by the retinal cells of the inner retina (i.e., bipolar, amacrine, or horizontal cells) although their exact origin remains debated [8, 9]. The OPs appear to be major contributors to the shaping of the ERG waveform [10] and there is an abundant literature attesting to the clinical value of including the OPs when analyzing pathological ERGs [6, 11, 12]. Unfortunately, in order to optimize the visualization of the OPs one must modify the recording bandwidth of the ERG from a broadband (e.g., 1–1000 Hz) to a narrower band (e.g., 100–1000 Hz) that removes the low-frequency components of the ERG (i.e., a- and b-waves) and consequently selectively enhances the high-frequency components (i.e., OPs) [2]. However, when doing so one must always keep in mind the possibility of introducing artifactual components (such as ringing artifacts and phase lags) to the ERG thus obtained.

It is clear from the above that the ERG waveform results from the amalgamation of several frequency components. Is it possible to monitor the frequency composition of the ERG signal without altering the signal as it is done with the bandwidth restriction approach? Would the use of such an approach significantly improve analysis of the ERG beyond what is accomplished when using time and amplitude measures of the ERG only?

Although advanced analytical approaches are now frequently used when studying biopotentials, such as the electroencephalogram [13], the electrocardiogram [14], and the electromyogram [15], to date they have only been sporadically applied to the ERG [8, 16, 17]. The purpose of this study was therefore to compare peak time and amplitude measurements of human photopic ERGs with measures obtained in the frequency domain using the Fourier analysis as well as in the time-frequency domain using the continuous (CWT) and discrete (DWT) wavelet transforms. For the sake of brevity, our study was limited to the photopic ERG only.

5. Materials and Methods

Normal photopic ERGs were obtained from 40 healthy subjects (26 females and 14 males, average age 29.9 ± 8.4 years) using a protocol that was approved by the Institutional Review Board of the Montreal Children's Hospital and in accordance with the Declaration of Helsinki.

According to a previously published method of ours, the ERGs were recorded with both eyes dilated (tropicamide 1%) using an active electrode (DTL fiber electrode) placed in the inferior conjunctival bag, with reference and ground electrodes pasted at the external canthi and forehead, respectively [18–21]. The ERGs were evoked to flashes of white light (flash duration: 20 μ s; interstimulus interval: 1.5 s; and average of at least 10 flashes per recording) of 0.64 log cd·s·m⁻² in intensity that were delivered against a rod desensitizing background light of 30 cd·m⁻² (measured using a research radiometer IL1700; International Light, Newburyport, MA, USA). ERG waves from both eyes were averaged to yield a single waveform of 150 ms in length (sampling rate: 3413.33 Hz) that included a prestimulus baseline of 20 ms.

5.1 ERG Analysis

The amplitude of the a-wave was measured from the prestimulus baseline to the most negative trough of the ERG, while the amplitude of the b-wave was measured from the trough of the a-wave to the most positive peak of the ERG that followed the a-wave [2]. Peak times were measured from flash onset to the peak of the a- and b-waves [2]. Given

that these measures of the ERG are taken in the time domain, they will be referred to as time domain (TD) measurements.

Frequency domain analysis (or Fourier analysis (FA)) of the ERG was carried out using the fast Fourier transform (FFT) algorithm implemented in MATLAB R2013b (Mathworks, Natick, MA, USA) as follows:

$$X(k) = \sum_{t=0}^{N-1} x(t) e^{-i(2\pi/N)tk}, \qquad k = 0, 1, \dots, N-1,$$
(1)

where X(k) represents the FFT coefficients, x(t) denotes the raw ERG time-series, and N denotes the number of data points in x(t). Each FFT coefficient weighs the energetic contribution of a single frequency component to the signal so that a frequency spectrum can be illustrated by tracing X(k). Considering the size (512 data points) and sampling frequency (3413.33 Hz) of our ERG waveforms, we were able to compute the FFT coefficients for frequencies ranging between 0 and 1706.66 Hz in increments of 6.66 Hz (i.e., frequency resolution). However, given the limitation imposed by our recording bandwidth (1–1000 Hz) we limited our analysis to frequencies ranging between 0 and 300 Hz to safely avoid artifactual contamination (such as that predicted by the Nyquist-Shannon sampling theorem [22]).

In order to localize the energy content of the ERG in both time and frequency we computed, using MATLAB, the continuous wavelet transform (CWT) of selected ERGs as follows:

$$CWT(a,b) = \frac{1}{\sqrt{a}} \int_{-\infty}^{\infty} x(t) \Psi^*\left(\frac{t-b}{a}\right) dt, \qquad (2)$$

where CWT(a, b) represents the wavelet coefficients localized at scales *a* (frequency) and moments *b* (time), x(t) denotes the unprocessed ERG time-series, and Ψ^* denotes the complex conjugate of the Morse wavelet [23], which was chosen for its good frequency resolution [24]. To illustrate the time-frequency scalogram of the CWT, we took the absolute value of CWT(a, b) and normalized it to its maximal value so that the timefrequency scalograms of the ERG are shown as colored two-dimensional plots of CWT(a, b) in which minimum energy values are displayed in blue and maximal values in red.

Use of the CWT approach allowed us to analyse the ERG, continuously, at every possible scale *a* and translation *b*. This approach, however, requires extensive computation time and also yields a lot of redundant information (i.e., since each coefficient has similar neighboring values) that will remain unused in the set of coefficients CWT(a, b) [25]. Interestingly, if the scale and translation parameters of the wavelet are taken at discrete values, we then obtain a discrete wavelet transform (DWT), where the scales *a* and translations *b* are based on powers of two (i.e., $a_j=2^j$, $b_{j,k} = k2^j$) so that (2) can be discretized as follows:

$$DWT(j,k) = \int_{-\infty}^{\infty} x(t) 2^{-j/2} \Psi(2^{-j}t - k) dt, \qquad (3)$$

where DWT(j,k) represents the wavelet coefficients localized at discrete scales *j* (frequency) and discrete moments *k* (time), x(t) designates the raw ERG time-series, and Ψ designates the Haar wavelet [25]. DWT(j,k) was computed using the fast wavelet transform algorithm of Mallat [25, 26] implemented in MATLAB. We chose the Haar wavelet, for its simplicity (i.e., simplest wavelet available) and its orthonormal basis, which allows the wavelet coefficients DWT(j,k) to be reconstructed accurately and efficiently without any loss of information (using the inverse DWT) even if all the redundant information contained in the CWT is discarded [25, 27]. Similar to the CWT scalograms, the most prominent energy component of the DWT scalogram will appear as a deep dark red (high energy) rectangle in the region of the DWT scalogram where it is located (i.e., located in time and frequency), and, conversely, the absence of any component at given locations will appear as deep dark blue (no energy) rectangles.

5.2. Statistical Analyses

Mean value, standard deviation (SD), and coefficient of variation (CV) were computed for all ERG parameters that were identified using the different analytical approach. Z-scores were used to evaluate the significance of selected descriptor changes. All tests were set to a level of significance of 5%.

6. Results

6.1. Time and Amplitude Measurements in the Time Domain

As reported in Table 1, time domain analysis allowed the identification of two major ERG components, one peaking at 13.53 ± 1.55 ms (mean \pm SD obtained in our 40 subjects) with an amplitude of $32.21 \pm 5.11 \,\mu\text{V}$ (identified as the a-wave at Figure 1(a)) and another one which peaks at 30.98 ± 1.33 ms with an amplitude of $104.81 \pm 18.66 \,\mu\text{V}$ (identified as the b-wave at Figure 1(a)).

6.2. Fourier Analyses (FA)

The frequency components contributing to the genesis of the ERG can be identified using the FA, such as that obtained with the FFT. This is best exemplified in Figure 1 and Table 1, where three major frequency components are identified in the normal ERGs. As shown with the black arrows, the low-frequency components of the ERG (presumably a- and bwaves) usually formed a smooth peak of large magnitude, culminating at 28.8 ± 5.7 Hz (i.e., mean \pm SD obtained from our 40 subjects) on the frequency spectrum. However, in some instances, two distinct peaks could be identified (see double black arrows in panels (a) and (d)). In the later cases, only the peak of highest magnitude was considered for further analysis. In contrast, the higher frequency components of the ERG (probably the OPs) usually formed two distinct peaks (see grey arrows) of low magnitude located at 75 \pm 7.7 Hz and 146 \pm 13.3 Hz, respectively. However, given that FA only looks at the frequency content of the ERG without taking into consideration if those frequencies are time-locked or not to the stimulus, its use can lead to erroneous interpretations of the ERG. This is best illustrated with the ERGs shown in Figures 1(c) and 1(d) where the noise contaminants (such as 60 Hz in Figure 1(c)) appear to contribute more to the making of the ERG than the retinal evoked components themselves. These limitations of FA can be overcome by adding a temporal resolution to the frequency domain.

6.3 Continuous Wavelet Transform (CWT) Analyses

As shown in Figure 2, use of the CWT approach allowed us to more precisely localize (in both time and frequency, as reported in Table 1) the abovementioned frequency components of the ERG signal and even in the presence of significant noise contaminants

(as seen in panels (c) and (d)). In each scalogram, the a- and b-waves formed a cluster of hot (dark red) coefficients (see white arrows) centered at 29.7 ± 5.7 Hz (i.e., mean \pm SD obtained in our 40 subjects) and peaking at 31.0 ± 0.9 ms (i.e., time-locked to the peak time of the ERG b-wave). Similarly, the OPs formed two distinct clusters (see grey arrows) centered at 73.8 ± 7.7 Hz and 150.3 ± 11.6 Hz and peaking at 32 ± 2.2 ms and 29.8 ± 1.9 ms, respectively. As shown in the scalogram of panels C and D, the high- (i.e., OPs) and low-frequency (i.e., corresponding to the a- and b-waves) components continued to remain the major light-evoked (i.e., time-locked to the stimulus) components of the ERG response in spite of significant noise contamination. The latter contrasts with results obtained using the FA where one cannot dissociate evoked from nonevoked frequency components (compare results shown in Figures 2(c) and 2(d) with corresponding FA results shown in Figures 1(c) and 1(d)).

In each scalogram of Figure 2, the value of the coefficients that identified the hot clusters pertaining to the a- and b-waves or OPs was centered on a section of the clusters where all coefficients had the same value (i.e., equipotential regions). This attribute of the CWT will generate redundant information (i.e., similar or equal coefficient values) that will complicate the accurate identification of the time-frequency coordinates (i.e., ill-posed coordinates) of the ERG components, by introducing an uncertainty factor. For example, given that the red clusters of Figure 2 extend over a large area of the scalograms, this prevents an accurate quantification of the a-wave energy, which is most probably hidden by the higher energy b-wave. These limitations can be overcome if we impose a discretization over the possible frequencies and times at which the information is computed.

6.4 Discrete Wavelet Transform (DWT) Analyses

As illustrated in Figure 3, the DWT scalograms decomposed the signals into seven contiguous frequency bands (20, 40, 80, 160, 320, 640, and 1280 Hz), each including a range of frequencies (reported in Table 1) around their respective central frequency (CF). For example, the 20 Hz band quantified the energy oscillating between 13.33 and 26.66 Hz (i.e., $20 \pm 20/3$, that is, CF ± CF/3). Use of this expansion simplifies the choice of relevant

coefficients (i.e., seen as rectangles of different sizes in the scalograms of Figure 3) that may be used as energy descriptors of the ERG.

6.5 Identification of DWT Descriptors

As seen with the DWT scalograms of Figure 3, the major frequency components are confined to the time-frequency region that is surrounded by white borders and where six major components can be identified (i.e., rectangles of various colors shown in panel (a) and magnified in panel (b)) and thus quantified (as reported in Table 1). The DWT also removed the redundancy so that the b-wave energy, quantified with the 20b and 40b descriptors (panel (b)), is now represented by two rectangles (i.e., identified as 20b and 40b) confined to an area of the scalogram that is limited to the vicinity of the b-wave peak rather than spread across most of the CWT scalogram. This allowed the quantification of two descriptors, time-locked to the a-wave, which we identified as 20a and 40a. The 20a and 40a were of lower energy compared to the 20b or 40b (see Table 1), indicating that, as expected, the b-wave energy is greater than that of the a-wave. Finally, the high-frequency components, indicated as 80ops and 160ops, were also easily identified (i.e., maximal value in the 80 and 160 Hz bands, resp.).

6.6 Improvement of ERG Segregation Using the DWT

As indicated above, the a- and b-wave components were seen on both the 20 Hz (20a and 20b descriptors) and 40 Hz bands (40a and 40b descriptors). These descriptors can be used to segregate ERGs of different morphologies. This is better illustrated in Figure 3(c), where two ERGs of distinct morphologies were similar (P > 0.05) in terms of a- and b-wave peak time and amplitude but were significantly different (P < 0.05) on the basis of their DWT b-wave descriptors (20b and 40b). In one example, the ascending limb of the b-wave has a sharp morphology (blue tracing) and showed a lower 20b descriptor (P < 0.05), compared to the broader ascending limb of the b-wave (compare first half of ascension of tracing in red) which disclosed an attenuated 40b parameter (P < 0.05). Similarly, we were also able to segregate ERGs that differed in OPs prominence (such as what is shown in Figure 3(d)). Although these ERGs were indistinguishable (P > 0.05) on the basis of peak time and amplitude measurements of the a- and b-waves, they were

significantly different (P < 0.05) on the basis of their 80ops and 160ops energy content which were higher (blue tracing) or lower (red tracing) compared to average.

6.7 Applications of Refined Analytical Approach to Clinical ERGs

In Figure 4(a) the ERG waveforms obtained from a patient diagnosed with retinitis pigmentosa (RP) that was followed up for more than 30 years are illustrated. As shown, the low amplitude (and low signal-to-noise ratio or low SNR) of these pathological ERGs, especially those obtained later in the disease process (tracings 23 and 29), seriously compromises an accurate measurement of these waveforms. This is best exemplified in Figure 4(c), where an accurate measurement of the b-wave amplitude could only be achieved for ERGs obtained within the first 13 years, due to the highly contaminated ERGs recorded subsequently. However, use of the DWT still permitted the extraction of b-wave descriptors (i.e., 20b and 40b) as shown in the scalograms of Figure 4(b) and therefore allowed us to monitor progression of the disease process for an additional period of 16 years, as shown in Figure 4(d). Furthermore, as revealed in Figure 4(d), both eyes followed the same degeneration pattern, which appeared to follow an exponential decay function that correlated well with that which characterized (using b-wave amplitude measures) the first 13 years. Interestingly, the use of the inverse DWT of the low-frequency bands (i.e., 20 and 40 Hz bands) allowed us to reconstruct noise-free ERGs (Figure 4(e)), that were nearly identical in both eyes, as it was also the case for the ERGs (measurable, high SNR) recorded in earlier exams (tracings 0, 1, 3, and 9 of Figure 4(a)). The validation of this denoising approach (i.e., inverse DWT) is further demonstrated in Figure 5, where we reconstructed the 20 consecutive single-flash recordings obtained from a RP patient that were used to generate the average waveform. As shown, the 20 denoised single-flash waveforms (red tracings) are nearly identical (mean (\pm SD) Pearson coefficients = 0.92 \pm 0.02) to the averaged response (i.e., blue tracing obtained by averaging the 20 consecutive noisy responses (gray traces)). In contrast, the mean Pearson coefficients obtained between the single sweeps and the averaged response were of 0.52 ± 0.03 .

7. Discussion

To date, analysis of the ERG relies mostly on time domain (TD) measurements (peak time and amplitude) of its two major components, namely, the a- and b-waves. However, as shown with the examples illustrated in Figures 4 and 5 and as previously suggested [28–31], TD measurements are subjected to noise contamination. These contaminants can arise from numerous factors such as the subject (e.g., eye blinks, head/eye movements, etc.), external sources (e.g., mechanical vibrations, electromagnetic coupling with the 50/60 Hz power lines, computer monitors, electrical lighting, etc.), and, if the data is digitized, the digitization process itself (e.g., digitization artifacts, aliasing, etc.). Therefore, limiting the ERG analysis to TD measures only could jeopardize the detection of subtle functional changes.

7.1 From Fourier to Wavelets

FA methods, such as the one presented in Figure 1 (i.e., FFT), performed well in identifying the three major frequency components of the normal photopic ERG response. However, when a noise contaminant was distributed over the entire ERG response, the resulting frequency-power distribution was misleading (as shown in Figures 1(c) and 1(d)). This is due to the fact that the FA assumes that all the frequency components that compose a signal are periodic and, consequently, ignores the possibility that some frequencies could be found at precise poststimulus time locations only. This explains why the amplitude of a component can be over- or underestimated when relying solely on FA analysis. In other words, while Fourier analyses are well-suited to identify the frequency components that compose the ERG signal, they are of no use to determine their respective magnitude and temporal location within the signal. Such information is of crucial importance if one wishes to define the signal with all its subtleties. This can be accomplished with a time-frequency domain analysis of the ERG.

Use of the CWT (Figure 2) approach allowed us to clearly identify the temporal as well as the frequency coordinates of the three major frequency components of the ERG, thus remedying the FA limitations alluded to above. We have shown that, with the CWT scalograms, each of these components was time-locked to the largest wave of the ERG
(i.e., the b-wave). Furthermore, compared to corresponding FA estimates, their respective weights (i.e., relative energy levels) were also more accurately determined, even in noisy ERG recordings.

7.2 The DWT: An Optimal Compromise

Interestingly, it seems, from FA and CWT analyses, that the components of the ERG cannot be associated with single frequency values but rather to a range of values. For example, in the FA power spectrums of Figure 1, each component had a broad Gaussian-like distribution (i.e., suggestive of a band of frequencies) rather than a sharp peak (i.e., suggestive of a single frequency) as it would be the case for a pure sinusoid. Similar broad distributions of individual frequency components were also observed in the CWT of the same ERG signals (see Figure 2), the major difference being that the magnitude of these frequency components can now be time-correlated to specific events of the ERG signal. Consequently, since these components contain a band of frequencies rather than a single frequency, analysis of the ERG at different frequency bands using the DWT scalogram (rather than at each possible frequency with the CWT) offers a simplified scheme (exempt of redundancy) to identify relevant ERG descriptors (see Figures 3(a) and 3(b)).

In the DWT scalograms the a- and b-waves were characterized by distinct components located in the 20 Hz (20a and 20b descriptors) and 40 Hz (40a and 40b descriptors) bands. It was difficult to accurately determine these distinct frequency components using the CWT, although in the FA some ERGs did show both the 20 and 40 Hz components (i.e., identified as double-peaks in Figures 1(b) and 1(d)). Furthermore, quantifying the ERG waveforms using the DWT augmented the specificity (i.e., ability to discriminate between distinct ERG morphologies, as shown in Figures 3(c) and 3(d)) and sensitivity (i.e., reduced the variability, as shown by the CV reported in Table 1) of the measures obtained.

Finally, at the end-stage of severe degenerative retinopathies (such as RP), nearly extinguished ERGs (e.g., low-SNR, such as the one shown in Figures 4(a) and 5) are often the last measurable signs of functional vision [32]. As shown in Figure 4, extracting relevant ERG descriptors from these residual ERGs using a TD approach (e.g., peak time

and amplitude measures) becomes nearly impossible as the SNR decreases. Use of the DWT permitted the quantification of such responses, thus extending the length of the follow-up period of disease progression by an additional 16 years. This allowed us to demonstrate the exponential decay known to characterize the long-term course of the cone ERG amplitude in patients affected with RP [33]. Furthermore, use of the inverse DWT of selected frequency bands allowed us to reconstruct noise-free ERG waveform thus confirming the presence of a residual biological response in signals that were reported as nonmeasurable using the TD approach. The validation of this DWT-denoising approach was demonstrated in Figure 5, where each of the 20 denoised ERGs was highly similar to the averaged response (obtained by averaging the 20 consecutive responses).

7.3 Limitations of the Study

In this study we limited the TD approach to its most widespread descriptors (i.e., amplitude and peak time of the a- and b-waves), but other unusual descriptors (e.g., areaunder-the-curve of the a- or b-wave, time to reach a certain percentage of the a- or b-wave amplitudes, steepness of the rising or descending flank of the b-wave, filtered OPs measurements, etc. [6, 31, 34]) could also be of use to identify subtle morphological changes, albeit similarly sensitive to noise contaminant errors.

8. Conclusion

In this paper, we have presented a brief overview of the different analytical approaches that can be used to quantify the ERG waveform. As long as the response remains measurable, the traditional measurements of the a- and b-waves can be used to monitor the peak time and the amplitude of the ERG signal. However, these measurements only look at the ERG signal as a whole, instead of looking at the different frequency components (possibly of distinct cellular origin) separately. The discrete wavelet transform offers the possibility to extract more components of the ERG signal, even in very poor SNR responses. Standardized time domain analysis of retinal function should thus be complemented with advanced DWT descriptors of the ERG. The latter should allow more sensitive/specific quantifications of ERG responses, facilitate follow-up of disease

progression, and identify diagnostically significant changes of ERG waveforms that are not resolved when the analysis is only limited to time domain measurements, thus bringing the analysis and interpretation of the ERG signal in the 21st century, as it is already the case with other biopotentials such as the electroencephalogram and electrocardiogram.

9. Acknowledgments

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11. Captions to Figures and Tables

Table 1: Normative data (mean ± standard deviation (SD) and coefficient of variation (CV, in bold)) obtained for each parameter (time, frequency, amplitude, power, and energy) assessed using the different analytical approaches compared in this study (time domain, frequency domain, and continuous and discrete time-frequency domain). The time domain allows timing and amplitude quantification of two major components (i.e., the a- and b-waves). The frequency domain identifies the frequency and power of three major components (probably associated with the a- and b-waves and OPs). The continuous time-frequency domain allows timing, frequency, and energy measurements of three main components (probably associated with the a- and b-waves and OPs). Finally, with the discrete time-frequency domain, the components are identified in predetermined temporal windows (i.e., intervals) and frequency bands (i.e., instead of precise timing and frequency) and allow more components to be identified and the a- and b-wave can be quantified independently (i.e., in contrast to the frequency domain or continuous time-frequency domain in which the a- and b-waves formed a single low-frequency component).

Figure 1: Fourier analysis (FA) of 4 normal ERGs. The frequency spectrums are shown as normalized power spectrum, in percentage, where the spectrums are normalized to their maximal value. The associated ERGs are shown at the bottom of each spectrum. The a-wave, b-wave, and OPs are indicated as "a," "b," and "OPs," respectively. (a) FA of a composite ERG, averaged from 40 subjects, showing the 3 typical frequency components that contribute to the ERG (~30 Hz: a- and b-waves contribution, black arrow; ~75 Hz and ~150 Hz: oscillatory potentials (OPs) contribution, gray arrows). (b) FA of an ERG showing enhanced OPs (increased ~75 Hz and ~150 Hz component contribution; thick gray arrows). (c) FA of a typical contaminated ERG showing the 3 standard ERG components (see arrows, same color-coding as previous panels) and a sharp, noise-related, maximal component seems to disturb the identification of the OPs component located at ~75 Hz. (d) FA of another contaminated ERG showing the 3 characteristic frequency components (see arrows, same color-coding as previous panels) of the ERG and 4 interference-related

components at 60, 120, 180, and 240 Hz, respectively (60-cycle harmonics contribution, red arrows). These noise components seem to complicate the identification of the two typical OPs components located at \sim 75 Hz and \sim 150 Hz.

Figure 2: Continuous Wavelet Transform (CWT) analysis of the ERGs that were shown at Figure 1. All scalograms were normalized to their maximal energy values, and the color-coding (see colorbar) indicates low (blue), moderate (green), and high (red) energy values. The associated ERGs are shown at the bottom of each scalogram. The a-wave, b-wave, and oscillatory potentials are indicated as "a," "b," and "OPs," respectively. (a) CWT of the composite ERG showing the energy (as per colorbar) and the temporal location of the 3 typical frequency components that were previously identified in the Fourier domain (~30 Hz: a- and b-waves contribution, white arrow; ~75 Hz and ~150 Hz: oscillatory potentials (OPs) contribution, gray arrows). (b) CWT of the ERG that had enhanced OPs [increased ~75 Hz and ~150 Hz energy; thick gray arrows]. (c) CWT of the contaminated ERG showing the 3 standard components (see arrows, same color-coding as previous panels) and a strip of moderate energy localized at 60 Hz for the whole duration of the signal (60-cycle interference contribution). (d) CWT of the second contaminated ERG also evidencing the 3 characteristic frequency components of the ERG and several transient, interference-related, patches of energy (60-cycle harmonics contribution).

Figure 3: Analysis method and classification improvement of normal ERGs using the discrete wavelet transform (DWT). (a) We computed the DWT of 40 normal ERGs with various signal-to-noise ratios (SNR = 13 ± 10 , gray traces; averaged response: red trace) and extracted, in each scalogram, descriptors (see panel (b)) localized inside the region of maximal energy (delimited by white borders). (b) Magnification of the averaged ERG (red trace at panel (a)) scalogram that shows where we identified six novel DWT descriptors of the ERGs (20a and 40a: a-wave energy; 20b and 40b: b-wave energy; and 80ops and 160ops: oscillatory potentials (OPs) energy). ((c) and (d)) The DWT descriptors were used to segregate ERGs of distinct morphologies, that had similar (P > 0.05) a- and b-wave amplitudes and peak times but significantly (P < 0.05) smaller 20b or 40b descriptors

(panel (c), white arrows) or significantly larger or smaller 80ops and 160ops descriptors in the DWT scalograms (panel (d), white circles).

Figure 4: (a) ERG traces (averaged from up to 100 responses) obtained at seven time points in the right (OD) eye and left (OS) eye of a male patient affected with retinitis pigmentosa (both eyes presented with nonrecordable scotopic ERGs, constricted visual fields, pigmentary deposits, and decreased visual acuity) in a time span of 3 decades. The horizontal (time) and vertical (voltage) scale bars apply to both eyes and some traces have been magnified ($\times 2$, $\times 5$, or $\times 10$ times) for visualization purposes. The flash onset is indicated by the black vertical arrow. ERG progression is shown in years since the first visit on the left-hand side. (b) Scalograms computed for each pathological ERG waveform (presented in the same order than in panel (a)) in which we quantified the 20b and 40b descriptors. Note that, in some scalograms, the position of the 40b descriptors was delayed (i.e., delayed latency of the b-wave) compared to normals (see Figure 3) and the 40b descriptors were always more severely attenuated than the 20b. (c) Progression of the TD b-wave amplitudes from both eyes. Because of the noise contaminants the 4th and 5th ERG were imprecisely measured (indicated by the lighter gray background on the graph), while the last two ERGs were nonmeasurable, thus preventing the quantification of disease progression from that point (indicated by the darker gray background). (d) Using the DWT descriptors of the b-wave (20b + 40b) allowed us to monitor the disease progression more precisely and for the whole time span (additional 16 years of monitoring: see zoomed box). (e) Using the inverse DWT, we reconstructed the low-frequency bands (i.e., 20 and 40 Hz bands), obtaining the biological denoised responses which are shown, in red, on top of the unprocessed gray tracings.

Figure 5: 20 consecutive single-flash ERG responses (gray traces) obtained from a patient affected with retinitis pigmentosa. The average of these 20 raw ERG responses canceled the uncorrelated noise to yield the blue tracing (overlaid on top of each single-flash response). DWT denoising of the individual noisy single-flash responses reveals denoised biological responses, which are shown as red traces. All red traces are nearly identical (shape and amplitude) to the trace obtained from the average (i.e., blue traces) of the 20

noisy responses, thus validating this denoising approach. The horizontal (time) and vertical (voltage) scale bars apply to each trace. The flash onset is indicated by the black vertical arrow.

12. Figures and Tables

Table 1:

	Time domain (peak time and amplitude)			
	Time (ms)	Frequency (Hz)	Amplitude (µV)	Origin
Main component 1	13.53 ± 1.55 (11)	n/a	32.21 ± 5.11 (16)	a-wave
Main component 2	30.98 ± 1.33 (4)	n/a	104.81 ± 18.66 (18)	b-wave
	Frequenc	y domain (Fourier ana	lysis)	
	Time (ms)	Frequency (Hz)	Power (µW/Hz)	Probable origin
Main component 1	n/a	28.81 ± 7.17 (29)	10.46 ± 2.15 (21)	a- and b-waves
Main component 2	n/a	75.38 ± 7.69 (10)	3.43 ± 0.89 (26)	OPs
Main component 3	n/a	146.00 ± 13.31 (9)	1.88 ± 0.43 (23)	OPs
	Time-frequency do	omain (continuous wav	elet transform)	
	Time (ms)	Frequency (Hz)	Energy $(\mu V \cdot s)$	Probable origin
Main component 1	31.04 ± 0.89 (3)	29.70 ± 5.71 (19)	202.8 ± 21.08 (10)	a- and b-waves
Main component 2	32.04 ± 2.22 (7)	73.81 ± 6.28 (9)	111.57 ± 23.17 (21)	OPs
Main component 3	29.81 ± 1.96 (7)	150.28 ± 11.57 (8)	69.29 ± 16.79 (24)	OPs
	Time-frequency	domain (discrete wave	let transform)	
	Time interval (ms)	Frequency band (Hz)	Energy $(\mu V \cdot s)$	Probable origin
Main component 1	-20 to 17.5	20 ± 6.6	78.08 ± 10.66 (14)	a-wave
Main component 2	17.5 to 55	20 ± 6.6	214.46 ± 19.02 (9)	b-wave
Main component 3	0 to 17.5	40 ± 13.3	100.79 ± 9.39 (9)	a-wave
Main component 4	17.5 to 55	40 ± 13.3	228.41 ± 28.05 (12)	b-wave
Main component 5	8.125 to 55	80 ± 26.6	145.83 ± 21.75 (15)	OPs
Main component 6	17.5 to 40.35	160 ± 53.3	80.81 ± 12.48 (15)	OPs

Figure 1:



Figure 2:



Figure 3:



Figure 4:



Figure 5:



1. Preface to chapter III.

In our first study (Chapter II), we compared the traditional time domain analysis to three advanced approaches and identified which technique was best suited for ERG analysis. The discrete wavelet transform provided more reproducible descriptors, which were able to distinguish between different ERG morphologies and to follow-up disease progression for longer time periods. This first study allowed us to conclude that the DWT offered the best compromises between time and frequency resolutions and was therefore the best method to study the ERG. However, in the latter study, our analyses were solely limited to the suprathreshold photopic ERG response obtained using one single optimal stimulus intensity. It is well known that the flash intensity and/or pathology may reduce or remove some ERG components, which when added together, contribute to the ERG waveform morphology. This aspect of photopic ERG genesis was not explored in our first paper. In this second study (Chapter III), we have used the DWT to decompose ERGs of several morphologies into their various time-frequency components. Normal human photopic ERGs evoked to a large range of stimulus intensities (to modulate the ERG waveform morphology) were analyzed using our novel DWT descriptors. The latter permitted us to evaluate if the use of DWT descriptors could be generalized to detect changes in ERGs, irrespective of their timing, amplitude, and morphology. Luminanceresponse curves that were generated using the various DWT descriptors revealed distinct, but co-varying, luminance-dependence patterns, demonstrating, for the first time, that the stimulus luminance differently modulates the different time-frequency components of the ERG. Defining the shape of the ERG so that subtle, physiologically-driven, morphological changes can be systematically and reproducibly detected remains a challenging problem, but the DWT could definitely meet this challenge. Additionally, analyses of ERGs obtained from patients affected by ON or OFF retinal pathway anomaly revealed that certain timefrequency components can be specifically associated with the function of the ON and OFF cone pathway. Therefore, this second study demonstrates that the DWT offers reproducible, physiologically meaningful and diagnostically relevant descriptors of the ERG over a broad range of signal amplitudes and morphologies.

2. Manuscript Title.

CHAPTER III

Manuscript 2 (Published Original Research Article)

FUNCTIONAL DECOMPOSITION OF THE HUMAN ERG BASED ON THE DISCRETE WAVELET TRANSFORM

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3. Abstract

The morphology of the electroretinogram (ERG) can be altered as a result of normal and pathological processes of the retina. However, given that the ERG is almost solely assessed in terms of its amplitude and timing, defining the shape of the ERG waveform so that subtle, physiologically driven, morphological changes can be systematically and reproducibly detected remains a challenging problem. We examined if the discrete wavelet transform (DWT) could meet this challenge. Normal human photopic ERGs evoked to a broad range of luminance intensities (to yield waveforms of various shapes, amplitudes, and timings) were analyzed using DWT descriptors of the ERG. Luminance-response curves that were generated using the various DWT descriptors revealed distinct (p < 0.05) luminance-dependence patterns, indicating that the stimulus luminance differently modulates the various time-frequency components of the ERG and thus its morphology. The latter represents the first attempt to study the luminance-dependence of ERG descriptors obtained with the DWT. Analyses of ERGs obtained from patients affected with ON or OFF retinal pathway anomalies were also presented. We show here for the first time that distinct time-frequency descriptors can be specifically associated to the function of the ON and OFF cone pathway. Therefore, in this study, the DWT revealed reproducible, physiologically meaningful and diagnostically relevant descriptors of the ERG over a wide range of signal amplitudes and morphologies. The DWT analysis thus represents a valuable addition to the electrophysiologist's armamentarium that will improve the quantification and interpretation of normal and pathological ERG responses.

4. Introduction

The electroretinogram (ERG) represents the biopotential generated by the retina in response to a light stimulus. To date, the ERG remains the only mean to objectively and noninvasively assess the functional integrity of the retina. Although interpretation of the ERG relies mostly on peak-time and amplitude measurements (i.e., usual time domain [TD] measurements) of the a- and b-waves (McCulloch et al., 2015), it is well known that retinal disorders can also alter the morphology of the signal, a feature of the ERG that is not objectively measured. This is best exemplified with the ERG of patients affected with congenital stationary night blindness (CSNB) which is often qualitatively reported as having a square-wave-like a-wave and truncated b-wave morphology (Heckenlively, Martin, & Rosenbaum, 1983; Lachapelle, Little, & Polomeno, 1983; Miyake et al., 1997). This morphological feature is pathognomonic for CSNB, but less obvious changes of the ERG morphology are likely to be unnoticed and/or ignored due to the nonexistence of more objective ERG shape-dependent measurements. Consequently, the present work addresses the following question: Is it possible to objectively quantify ERG signals of various shapes so that, physiologically driven, morphological changes can be systematically and reproducibly detected and used as diagnostic clues?

Intuitively, the morphology of a given ERG waveform is most likely determined by how its different frequency components combine, in energy and latency, to generate the waveform. While different frequency components of the ERG signal can be identified using Fourier analysis (such as the fast Fourier transform [FFT]), they cannot be localized in time because the FFT does not include a temporal resolution (Breslin & Parker, 1973; Gur & Zeevi, 1980; Poppele & Maffei, 1967). Both spectral and temporal resolutions are needed to accurately define the properties (e.g., morphology) of transient time-varying signals (such as the ERG) and, fortunately, time and frequency resolutions can be optimally obtained with the use of continuous (CWT) or discrete (DWT) wavelet transforms (Mallat, 2009). The growing use of these modern approaches in the fields of auditory, somatosensory, and event-related evoked potentials has led to significant diagnostic improvements. For example, early work by Thakor, Guo, Sun, and Hanley (1993) revealed that the wavelet decomposition was well suited to characterize and rapidly detect complex changes in the shape of somatosensory evoked-potentials that resulted from cerebral hypoxic injury. Similarly, Demiralp and Ademoglu (2001) also went beyond traditional TD amplitude and latency measurements and beyond Fourier analysis by processing an extensive characterization of event-related potential (ERP) signal morphologies through the use of the wavelet transform. They also demonstrated that the waveform morphology of the ERP signal was carrying important information that could be segmented in multiple functional components by the wavelet decomposition (Demiralp & Ademoglu, 2001). In another study, Quiroga and Garcia (2003) improved the detection of single-trial ERPs using a wavelet-denoising approach that selectively reconstructs the most meaningful DWT coefficients within precise frequency bands and time windows (Quiroga & Garcia, 2003). Given the better performances of their algorithm, compared to more common filtering approaches, their technique subsequently inspired several investigators.

In contrast, fewer attempts have been made to evaluate the usefulness of wavelet techniques to the ERG. So far, wavelets were sporadically applied to the analysis of pattern ERG (PERG), multifocal ERG (mfERG) and full-field scotopic ERGs of human and rats. In rats, CWT analysis revealed that two frequency components (i.e., 70-80 Hz and 120-130 Hz) contributed to the genesis of the oscillatory potentials (OPs; Forte, Bui, & Vingrys, 2008). This study also showed that the energy level, the frequency and the latency of the two OP frequency bands had distinct luminance-response (LR) functions, suggesting that they might be evoked by different retinal elements/mechanisms. The CWT also revealed that the scotopic a-wave of normal human subjects was composed of three frequency components (20, 140, and 180 Hz) and that the higher frequency component was absent in achromates, suggesting that the CWT could also help in the diagnosis of photoreceptoral diseases (Barraco, Persano Adorno, & Brai, 2011). Moreover, DWT coefficients were shown to be superior to traditional TD measures in segregating normal and pathological PERG waveforms using principal components analysis (Rogala & Brykalski, 2005). It was also shown that it is possible to approximate the TD a-wave, b-wave, and at least one OP using the inverse DWT of the scotopic ERG waveform (Varadharajan, Fitzgerald, & Lakshminarayanan, 2007). Similarly, Miguel-Jiménez, Boquete, Ortega, Rodriguez-Ascariz, and Blanco (2010) used the DWT decomposition to reconstruct mfERG waveforms into different frequency bands and suggested that this approach could potentially be more sensitive to detect changes in glaucoma patients, compared to Humphrey visual field tests. As indicated above, wavelet analysis can reveal subtle (and possibly diagnostic) ERG changes that are almost impossible to appreciate with TD measures.

Of interest, we previously compared the FFT, CWT, and DWT with the more traditional TD measures (amplitude and peak time of the a- and b-waves) in their abilities to analyze the ERG signal and concluded that the DWT offered significant advantages (Gauvin, Lina, & Lachapelle, 2014). With its simplified scalograms and its predetermined time-frequency borders assigned to each ERG component, the DWT significantly eases the identification of relevant descriptors and highlights additional frequency components. However, in the latter study of Gauvin et al. (2014), analysis was limited to the suprathreshold photopic ERG response, which was previously shown to be the most complete (optimal) cone-mediated ERG response (Lachapelle, Rufiange, & Dembinska, 2001). Of course, it is also well known that flash intensity and/or pathology may reduce or remove some of the components, which when added together, make this optimal photopic ERG signal, an aspect of photopic ERG genesis that was not explored in our first paper, thus preventing us from evaluating if the use of DWT descriptors can be generalized to detect changes in ERGs, irrespective of their morphology, amplitude, and timing.

As formerly demonstrated, the LR function of the human photopic ERG b-wave is rather unique in that, with a progressive increase in stimulus luminance, the amplitude of the b-wave first increases, reaches a maximal value and then decreases before reaching a plateau with the brightest intensities. This change in amplitude is always accompanied by variations in the overall ERG waveform morphology. This phenomenon, first described by Wali and Leguire (1992) as the photopic hill (PH), was previously explored by the current authors (Garon et al., 2010; Rufiange et al., 2003; Rufiange, Dumont, & Lachapelle, 2005; Rufiange, Rousseau, Dembinska, & Lachapelle, 2002) and others (Hamilton, Bees, Chaplin, & McCulloch, 2007; Kondo et al., 2000; Ueno, Kondo, Niwa, Terasaki, & Miyake, 2004). Normal ERG responses evoked to 21 stimulus intensities that covered a range of 5 log-units of intensity (to generate the PH) were therefore used herein as a method to modulate the morphology, amplitude, and timing of the ERG response.

The current study aims to characterize and compare the luminance-dependence of the various DWT descriptors that were used to analyze the morphologically different ERGs known to compose the normal PH. We hypothesize that the ERG morphology will be captured by the DWT descriptors and that PH-like LR functions will also characterize LR functions obtained using DWT descriptors of the ERG that are concomitant with the bwave. Moreover, we investigated the complexity of the ERG waveform using new DWT descriptors that we derived from wavelet variance analyses (WVAs). Finally, to assess if DWT descriptors appraise meaningful physiological attributes of the cone ERG, analyses of abnormal responses obtained from patients affected with known retinal pathway anomalies are also presented.

5. Methods

Analyses were conducted on a total of 25 (16 women and nine men) normal subjects $(30.5 \pm 8.1 \text{ years old})$ who had signed an informed consent form previously approved by the Institutional Review Board of the Montreal Children's Hospital. Experiments were conducted in accordance with the Declaration of Helsinki. This study was limited to the photopic ERG for the uniqueness of its LR function (the so-called PH), with its four characteristic phases (i.e., ascent, maximal value, descent, and final plateau phases) and ensuing different cone ERG morphologies (Garon et al., 2010; Hamilton et al., 2007; Kondo et al., 2000; Rufiange et al., 2003; Rufiange et al., 2005; Rufiange et al., 2002; Ueno et al., 2004; Wali & Leguire, 1992).

5.1 Preparation of subjects and ERG recordings

According to a previously used protocol of ours (described in Garon et al., 2010; Rufiange et al., 2003; Rufiange et al., 2005; Rufiange et al., 2002), ERG signals were recorded (LKC UTAS-E-3000 system; LKC Technologies Inc., Gaithersburg, MD) with both eyes dilated (Tropicamide 1%) using a Dawson, Trick, and Litzkow (DTL) fiber electrode (27/7 X-Static silver-coated conductive nylon yarn, Sauquoit Industries, Scranton, PA) placed in the inferior conjunctival bag, with reference and ground electrodes (Grass gold-plated cup electrodes filled with Grass EC2 electrode cream; Grass Technologies, Warwick, RI) pasted at the external canthi and forehead, respectively. Photopic ERGs (bandwidth: 1–500 Hz; amplification: 20,000×; attenuation: 6 dB; sampling frequency: 3413.33 Hz) were recorded against a broadband white (color temperature: 6500 K), rod-desensitizing, background light of 30 cd.m⁻². Photopic hills were obtained in response to graded intensities of stimulation (ranging between –0.8 and 2.64 log cd.s.m⁻² in 14 steps of ~0.26 log-units; average of 10 flashes per intensity; flash duration: 20 μ s; white light; interstimulus interval: 1.5 s; prestimulus baseline: 20 ms) in 15 of the 25 subjects. ERGs evoked to seven dimmer flash intensities (ranging between –2.23 and –1.00 log cd.s.m⁻² in 0.2 log-unit steps; average of 50 to 300 flashes; flash duration: 20 μ s; white light; interstimulus interval: 0.3 s; prestimulus baseline: 20 ms) were obtained from the other 10 subjects. Background light and integrated flash luminances were measured with a research radiometer (IL1700; International Light, Newburyport, MA). ERGs from both eyes were averaged to yield a single waveform and imported in MATLAB R2014a software (Mathworks, Natick, MA) for further analyses.

5.2. Selection of pathological ERGs

Depending on the nature of the disease process, retinal disorders can alter the amplitude and/or the peak time of the photopic and/or scotopic ERGs. Moreover, retinopathies can also remarkably affect the overall morphology of the ERG waveform. For the sake of the current work, we chose to limit our analysis of pathological signals to photopic (flash intensity: $0.64 \log \text{ cd.s.m}^{-2}$; rod-desensitizing background light: 30 cd.m^{-2}) ERGs obtained from patients (n = 20) affected with Type-1 CSNB (n = 10) or congenital postreceptoral cone pathway anomaly (CPCPA; n = 10). These patients were selected for two main reasons. Firstly, the selected ERG waveforms show, upon visual inspection, strikingly different morphological features that, we claim, will be captured by the DWT descriptors. Secondly, the functional anomaly of patients affected with CSNB is known to specifically reside with the ON-pathway (based on electroretinographic (Langrova et al., 2002; Miyake, Yagasaki, Horiguchi, & Kawase, 1987; Quigley et al., 1996) and molecular (Bech-Hansen et al., 2000; Dryja et al., 2005; Gregg et al., 2007; Pusch et al., 2000) findings and complementary, the functional anomaly of patients affected with CPCPA is believed to lie on the OFF-pathway (Garon et al., 2014; Lachapelle et al., 1998). We

hypothesize that retinal conditions affecting the ON (CSNB) and OFF (CPCPA) pathways will affect different DWT descriptors.

5.3. TD analysis of the ERG

The amplitude of the a-wave was measured from the baseline to the trough of the a-wave and that of the b-wave from the trough of the a-wave to the peak of the b-wave. The amplitude of each OP was measured from trough to peak and summed to obtain the sum of OPs (SOPs). We also computed the signal-to-noise ratio (SNR) of selected ERGs as the ratio of the b-wave amplitude over the amplitude of the baseline noise (i.e., maximal peak-to-peak amplitude measured within the first 20 ms of recording prior to flash onset).

5.4. Computation of the DWT

The DWTs of all ERG responses were obtained using the Matlab R2014a application (computation detailed in Appendix A). For each DWT, we displayed eight levels (i.e., eight frequency bands) of decomposition, each with a distinct central frequency (Level 1 = 1280 Hz; Level 2 = 640 Hz; Level 3 = 320 Hz; Level 4 = 160 Hz; Level 5 = 80 Hz; Level 6 = 40 Hz; Level 7 = 20 Hz; and Level 8 = 10 Hz; see Figure 1A), where each band quantified the contribution of a range of components oscillating around the central frequency (for example: $20 \text{ Hz} = 20 \pm 20/3 \text{ Hz}$, $40 \text{ Hz} = 40 \pm 40/3 \text{ Hz}$, and so on).

5.5. DWT quantification of the ERG waveform with local wavelet maxima descriptors

DWT scalograms were obtained by plotting the absolute value of the wavelet coefficients on a dyadic time-frequency grid. For this study, we have considered the following six LWM descriptors (identified in Figure 1A and defined in Table 1), namely 20a, 20b, 40a, 40b, 80ops, and 160ops (see Appendix A for details on how they were derived). As indicated in Table 1 (and illustrated in Figure 1A), each LWM descriptor quantified the energy of local oscillations of the ERG signal within a precise and predetermined time-frequency window. Due to their temporal positions (compare Figure 1A, B), we previously suggested (Gauvin et al., 2014) that the 20a, 40a, 20b, and 40b DWT descriptors were most probably associated with the a- and b-waves of the ERG signal, respectively. This claim will be further investigated herein by assessing if strong

correlations exist between the LR functions of these DWT descriptors and that of the aand b-wave amplitude, respectively. Similarly, due to their frequencies (80–160 Hz) and temporal positions, the 80ops and 160ops descriptors were associated to the OPs. In this study, we needed the OPs descriptors to quantify all OPs that were included in a given ERG waveform, irrespective of stimulus intensity or health status of the retina. Consequently, as shown in Table 1, we computed the 80ops and 160ops descriptors as the average of five coefficients in order to obtain a global measurement of the OPs. Note that the white borders delimiting the 160 ops descriptor (Figure 1A) included 10 coefficients so, in order to have an unbiased average (i.e., to have the same number of coefficients to average for both the 80ops and 160ops), we computed the 160ops as the average of five coefficients (see details in Table 1 and its caption). As shown in Figure 1C, the summation of the four frequency bands (i.e., 20, 40, 80, and 160 Hz) that included the six LWM descriptors considered in the present study allows us to reconstruct a synthetic ERG waveform (i.e., inverse wavelet transform; last black tracing of Figure 1C) that explain 98.53% (as per Pearson coefficient) of the original ERG (blue tracing), indicating that the time-frequency components that we identified are the most (if not the only) important contributors to the genesis of the photopic ERG waveform. Finally, given that variation in the peak times of the different ERG components is expected and that the DWT is not a shift-invariant transform (Guo, 1995), we calculated each LWM as the maximum value obtained while shifting (i.e., translating) the complete ERG waveform to the left and right directions of the time axis. As reported in Table 1 (column 5), the magnitude of the translation was limited to a given range to the left and right (i.e., corresponding to positive and negative translation values in Table 1). The range of the translation was selected by trial and error in order to conservatively cover the expected variation of photopic ERG peak times (i.e., ~ 5 ms/log-unit increment of the stimulus intensity, as estimated from Garon et al., 2010). The above-mentioned translations thus allowed an optimal (i.e., maximal) measurement of the LWM more independently of ERG peak times. Full translation details and demonstrations are reported in Appendix A.

5.6 DWT quantification of the ERG waveform with WVA

WVA represents the variance of all wavelet coefficients obtained at each level (i.e., frequency band) of a DWT (Percival, 1995). As shown in Figure 1D, WVAs are reported as a plot correlating the variance values (expressed as the standard deviation in this study) with the corresponding DWT frequency level (Gallegati, 2008; Park & Willinger, 2000). As exemplified, the variance of a typical ERG evoked at 0.64 log cd.s.m⁻² starts at nearzero values (Levels 1 to 3), rapidly increases (steepest increment between Levels 5 and 6) to reach a maximum at Level 7, which is then followed by a final decrease in the value of the wavelet variance. This graphic representation allowed us to identify the delta-variance $(\Delta$ -variance) descriptor, the computation of which is presented in Appendix A and illustrated in Figure 1D. The Hölder exponent (also termed scaling exponent) represents another descriptor that can be computed using the WVA of DWT (Abry, Flandrin, Taqqu, & Veitch, 2002). This descriptor, which is amplitude-independent, characterizes the irregularity (or roughness) of the signal, a feature often associated to the complexity of a given waveform (Bishop, Yarham, Navapurkar, Menon, & Ercole, 2012; Sen, Litak, Kaminski, & Wendeker, 2008). The computation procedure of the Hölder exponent is detailed in Appendix A and illustrated in Figure 1E.

5.7 Selection of mother wavelets

The selection of a wavelet is mainly related to the choice of an optimal balance between temporal accuracy and spectral resolution to represent the fluctuations in the signal. This trade-off is controlled by the number of vanishing moments of the wavelet. High number of vanishing moments implies a better spectral resolution, but lesser temporal accuracy and vice-versa (Strang & Nguyen, 1996). In this study, we defined the local wavelet maxima (LWM) descriptors of the ERG to analyze features of the signal that are well-localized in time (such as the a- and b-wave features). With zero vanishing moment, the Haar wavelet provides the best temporal accuracy and hence, the Haar wavelet coefficients have the ability to optimally identify local features of a signal (Daubechies, 1992). Furthermore, the Haar wavelet has the shortest wavelet filter length, making its coefficients less sensitive to cross-contamination from neighboring oscillations (such as that of the b-wave on the a-wave). Given the above properties, we opted for the Haar wavelet to analyze the LWM descriptors of the ERG. In contrast, the WVA descriptors analyze global scaling characteristics of the signal. The symmetric Daubechies wavelet (Lina & Mayrand, 1995) set with two vanishing moments (or SDW2) also has a compact support, but because of its shape and added vanishing moments (i.e., better spectral resolution), it has the property to specifically encompass the entire ERG waveform. This makes this wavelet blind to low-order polynomial trends that are nonspecific to the ERG waveform, thus leading to a sparse representation of the genuine fluctuations present in the signal. The latter allows a more robust estimation of wavelet variance at each level of the DWT (Abry et al., 2002). The SDW2 wavelet was thus selected to quantify the WVA descriptors.

5.8 Statistical analysis

Statistical analysis of the LWM descriptors was performed using two-way withinsubjects analysis of variance (ANOVA; between -0.8 to 2.64 log cd.s.m⁻²; n = 15 subjects) followed by post hoc Bonferroni-paired t tests for multiple hypotheses testing with repeated measures. Statistical analysis of the wavelet variance descriptors (Hölder and Δ -variance) was obtained using one-way (stimulus intensity) within-subjects ANOVAs (between -0.8to 2.64 log cd.s.m⁻²; n = 15 subjects). Multiple comparisons (based on Bonferroni-paired t test for repeated measurements) were used to compare the values of selected pairs of means obtained under different stimulus luminances. The pathological ERG groups (CSNB and CPCPA) were compared to control and between them using unpaired two-sample t tests. The coefficient of variation (CV) of selected group data was computed as the standard deviation divided by the mean and multiplied by 100. Finally, Pearson correlation coefficients (i.e., r for rho) were used to compare the similarities between ERG waveforms or between LR functions. The significance level of each test was fixed at 0.05.

6. Results

Figure 2A illustrates representative photopic ERG responses, evoked to progressively brighter stimuli, which were used, in the present study, in order to generate the typical PH (Figure 2B). The corresponding DWT scalograms are shown in Figure 2C.

The LWM descriptors considered in the present study (identified in the scalogram representing the ERG signal evoked to the 0.39 log cd.s.m⁻² stimulus in Figure 2C and delimited with white borders in the other scalograms) show that, irrespective of the intensity of the stimulus, the maximal energy of the signal is always contained within a time-frequency region delimited by these descriptors. Therefore, irrespective of the amplitude of the ERG signal, LWM associated with the a-wave (20a, 40a), b-wave (20b, 40b) and OPs (80ops, 160ops) remained quantifiable (as per color scale). Of interest, as shown in Figure 2D, when the total LWM energy (i.e., Σ Energy = 20a + 40a + 20b + 40b + 80ops + 160ops) of each ERG waveform is plotted against the intensity of the stimulus, the shape of the resulting function is reminiscent of the PH obtained when only the amplitude of the b-wave is considered (as in Figure 2B)—both functions reaching their maximal values with the ERG evoked to the 0.39 log cd.s.m⁻² stimulation. This confirms that, as hypothesized, the PH-like shape (originally evidenced with TD measures of the b-wave; blue curve of Figure 2B) continues to remain a signature feature of the cone ERG LR function when the ERG response is quantified in the time-frequency domain.

6.1. Luminance-dependence of the LWM descriptors

LWM descriptors were computed using the Haar wavelet. Figure 3A and B report the mean (± 1 SD) photopic a- and b-wave LR functions obtained using the LWM descriptors weighing the a-wave (20a, 40a; Figure 3A) and b-wave (20b, 40b; Figure 3B) energy levels. As seen in Figure 3A, the 20a and 40a descriptors follow distinct LR patterns; the 20a descriptor first increases slowly (from -0.8 to 0.39 log cd.s.m⁻²) and then more abruptly (from 0.39 to 1.4 log cd.s.m⁻²) before reaching a plateau with the four brightest intensities. In contrast, the 40a descriptor appears to follow a logistic-like growth function. As shown in Figure 3D, the growth pattern obtained by summating the 20a and 40a DWT descriptors is almost identical to that obtained using TD measurements of the awave (Pearson's correlation coefficient = 0.9983). A similar decomposition of the b-wave (Figure 3B) reveals that the LR functions of the two frequency components (i.e., 20b and 40b) follow distinct PH-like shapes. First, the peak of the 20b is flat compared to the sharper peak of the 40b LR function, and second, while the maximal energy value for the 20b descriptor is reached with the 0.64 log cd.s.m⁻² stimulus, that of the 40b component is attained at 0.39 log cd.s.m⁻². Again, as shown in Figure 3E, the growth function obtained by summating the 20b and 40b DWT descriptors is nearly identical (Pearson's correlation coefficient = 0.9988) to that obtained using the TD measurements of the b-wave (the socalled PH shown in Figure 2B). Statistical analysis revealed that the interaction effects (frequency band \times stimulus intensity) were significant for both the a-wave (df = 13; F = 37.16; p < 0.00001) and b-wave (df = 13; F = 27.28; p < 0.00001). Of interest, post hoc analysis revealed that, depending on the intensity of the stimulus used, the energy level concealed in the 40 Hz a-wave parameter (40a) was either significantly higher (p < 0.05) or equal (p > 0.05) to that concealed in the 20 Hz a-wave parameter (20a). Similarly, the 40-Hz b-wave parameter (40b) was either significantly higher (p < 0.05), equal (p > 0.05), or significantly lower (p < 0.05) than the 20-Hz b-wave parameter (20b). These significant intensity-dependent differences (indicated by the black asterisks in Figure 3A, B) in the energy level of the LWM descriptors indicate that the intensity of the stimulus significantly modulates the time-frequency composition of the ERG. As shown in Figure 3C, a similar PH pattern was also obtained with the higher frequency components of the ERG (80ops and 160ops), both attaining maximal values with the 0.39 log cd.s.m⁻² stimulus. Statistical analysis revealed that the interaction effect (frequency band \times stimulus intensity) was significant (df = 13; F = 28.98; p < 0.00001). Post hoc tests indicated that irrespective of the intensity, the magnitude of 80 Hz OPs energy descriptor (80ops) was significantly higher (p < 0.05) than the 160 Hz (160ops) one. Finally, as shown in Figure 3F, the LR function obtained by summating the 80ops and 160ops descriptors covaried (Pearson's correlation coefficient = 0.9831) with that obtained using the TD measurement of the SOPs.

6.2. Luminance-dependence of the WVA descriptors

WVA descriptors were computed using the symmetric Daubechies wavelet. Represented in Figure 4A is the mean (± 1 SD) LR function of the Hölder exponent. With progressively brighter stimuli, the value of the Hölder exponent first increases, reaches a maximal value (e.g., maximal complexity of the ERG waveform) at 0.64 log cd.s.m⁻², which is then followed by a gradual decrease with brighter stimuli. The main effect of the stimulus intensity was found to be significant (df = 13, F = 51.26; p < 0.00001). Of note, though the Hölder exponent is amplitude-independent, its LR function is reminiscent of the PH obtained with TD measure of b-wave amplitude, which is shown in Figure 4C. There are, however, important differences, such as (a) the peak value is reached at slightly brighter stimulus intensity (i.e., 0.64 compared to 0.39 log cd.s.m⁻²) and (b) the absence of a plateau effect with the brightest stimuli (compare Figure 4A, C).

Similarly, as shown in Figure 4B, the LR function of the Δ -variance parameter also adopts a PH-like pattern that reaches its maximal value with the 0.64 log cd.s.m⁻² flash and does not plateau with the brightest stimuli. This main effect of the stimulus intensity was also found to be significant (df = 13, F = 22.54; p < 0.00001).

Of interest, although the LR functions of the two WVA descriptors (Hölder and Δ -variance) reached their peak value with the ERG evoked to the 0.64 log cd.s.m⁻² stimulus, the shapes of the resulting LR functions differed significantly; the peak of the Hölder exponent function being smoother compared to the sharper peak of the Δ -variance function. Supportive of the latter, post hoc analysis revealed that Hölder values immediately adjacent (i.e., evoked at 0.39 and 0.9 log cd.s.m⁻²) to the peak value (0.64 log cd.s.m⁻²) were not significantly different from this peak value (p > 0.05), while the peak value of the Δ -variance descriptor was significantly larger (p < 0.05) than that of the values from the two neighboring intensities.

6.3 DWT analysis of ERGs of lower amplitudes

Figure 5A illustrates photopic ERGs evoked to flash intensities ranging between -2.23 and $-1.0 \log \text{ cd.s.m}^{-2}$, while the corresponding DWT scalograms are presented at the right-hand side of each waveform. As shown in Figure 5B, the amplitude of the a- and b-waves thus obtained grows progressively (a-wave: from 0.6 to 5.0 μ V; b-wave: from 0.9 to 10.75 μ V) with brighter stimuli to reach, in response to the $-1 \log \text{ cd.s.m}^{-2}$ stimulus, values that are slightly lower (a-wave: 5.0 μ V; b-wave: 10.75 μ V) than values obtained at $-0.8 \log \text{ cd.s.m}^{-2}$ (a-wave: 7.41; b-wave: 18.24 μ V; e.g., Figure 2A, B). Similar to what was shown with the larger amplitude ERGs (see Figure 2C), DWT scalograms of the low-voltage ERGs also reveal that the maximal energy of the ERG signal remains localized in the time-frequency region that is delimited with the six LWM descriptors (white borders) defined above (i.e., 20a, 20b, 40a, 40b, 800ps, 1600ps). Similarly, each DWT descriptor

(Figure 5C through F) grows progressively with brighter stimuli to reach, in response to the $-1 \log \text{ cd.s.m}^{-2}$ stimulus a value slightly lower than the value obtained at $-0.8 \log \text{ cd.s.m}^{-2}$ (see Figures 3 and 4), confirming that DWT descriptors can be used to monitor the ERG over a wide range of amplitudes (i.e., in this study: from less than 1 μ V to more than 100 μ V).

In addition, although intuitively one would expect more variability in the quantification of the different ERG parameters as the ERG response becomes noisier (i.e., lower SNRs; compare Figure 6A, B), it seems that this variability impacts more on TD parameters compared to DWT ones. This is best illustrated with the data shown in Figure 6C, comparing the CV of the a- and b-wave amplitudes measured using the TD approach (TD a-wave and TD b-wave) with their equivalent measurements obtained using the DWT (DWT 20a + 40a; DWT 20b + 40b) for ERGs evoked to $-2.23 \log cd.s.m^{-2}$ (mean SNR in the TD: 1.62 ± 0.82) and 0.64 log cd.s.m⁻² (mean SNR in the TD: 19.65 ± 6.77). A 92% reduction in the SNR values, increased (i.e., seen from right to left in Figure 6C) the CV of the a- and b-wave measurements by 125% and 98%, respectively, using the TD approach, compared to 50% and 31% using the DWT approach.

6.4 DWT analysis of ERGs from patients affected with ON or OFF pathway anomaly

Results presented above demonstrate that selected DWT descriptors follow significantly different LR functions when evoked to progressively brighter stimuli, such as the 40b energy (Figure 3B) being significantly higher than that of the 20b for the rising part of the PH (i.e., dimmest stimuli), and conversely, the 20b being significantly higher than the 40b for the descending part of the PH (brightest stimuli), thus suggesting that they might quantify distinct physiological processes (or pathways) of the retina. To further investigate this claim, ERGs of patients affected with known retinal pathway anomalies, specifically affecting the ON or OFF pathway, were analyzed using the DWT.

This is best exemplified in Figure 7A, which shows representative ERG tracings obtained from patients affected with Type-1 CSNB (specific ON-pathway anomaly; Tracings 2, 3, and 4) or CPCPA (specific OFF-pathway anomaly; tracings 5, 6 and 7). As can be seen, the ERG waveform morphology of these patients is strikingly different from

that of a normal control (Tracing 1) and between them (compare Tracings 2, 3, and 4 with Tracings 5, 6, and 7). As a result, several of their DWT descriptors differed significantly. This is best evidenced with the accompanying group data and statistics reported in Table 2. As shown, the Hölder exponent was the most affected DWT descriptor in both groups and was found to be as low as 6.6 and 8 SDs below the control value for CSNB and CPCPA, respectively. Of note, out of the 10 descriptors presented in Table 2, it is also the Hölder exponent that had the least variability in control subjects (CV of 4.14%), which could explain its superior sensitivity to pathological changes. Moreover, from a TD point of view, the amplitude of the a- and b-waves (TD a and TD b parameters in Table 2) of both patient groups was significantly reduced (apart from the normal a-wave amplitude found in CPCPA patients). However, these parameters were only the fifth and sixth most affected descriptors (for the TD b parameter of CPCPA and CSNB, respectively), suggesting that these traditional ERG parameters are less sensitive than DWT ones (maximum of 1.2 and 2.5 SDs below the mean for TD a and TD b, respectively, compared to a maximum of 8 SDs below the mean for the Hölder exponent).

Significant differences were also found between the two patient groups, the most important ones being that of the 20b and 40b descriptors, which had a percent difference of 129.42% and 101.39% between the two groups, respectively. These DWT differences are better illustrated in the bar graphs presented in Figure 7B and C. As shown, the 20b (Figure 7B) and 40b (Figure 7C) descriptors were more specifically reduced (by 2.8 folds and 3.0 folds; p < 0.00001) in CSNB and CPCPA, respectively. As a result, while in control the 40b-to-20b ratio (i.e., 40b divided by 20b; shown in Figure 7D) was found to be almost unity (1.05 ± 0.06), that of CSNB patients was found to be of 2.01 ± 0.30 (i.e., 40b > 20b), and that of CPCPA of 0.43 ± 0.06 (i.e., 20b > 40b). Use of this ratio significantly reduced intersubject variability (CV of control is 5.71% for 40b-to-20b ratio compared to 18.18% and 14.92% for 20b and 40b, respectively) and significantly emphasized the effect size seen between the two patient groups (% difference = 367.44% for 40b-to-20b ratio compared to 129.42% and 101.39% for 20b and 40b, respectively).

It is clear from the above that DWT descriptors can be specifically or differently affected by a given disease process and, consequently, that they could be used to highlight meaningful differences between various ERG responses. Clearly, the data shown in Figure 7 and Table 2 provide interesting demonstrations of how DWT analysis could complement the traditional analysis of the ERG by offering an alternative quantification, which could better reflect the (presumably) unlimited ways by which retinal function (as reflected with the ERG) can be modulated as a consequence of normal (Figures 3 and 4) and pathological processes (Figure 7).

7. Discussion

Reproducible LWM, time-locked with the a-wave, b-wave, and OPs were identified in high- (>100 μ V; Figures 3 and 4) and low- (<1 μ V; Figure 5) voltage ERGs, a feature of diagnostic relevance especially if one wishes to use the ERG to monitor disease progression in severe degenerative retinopathies (such as Retinitis Pigmentosa), whose final outcome is often characterized by very low-amplitude ERGs, or even extinguished ERGs (Rispoli, Iannaccone, & Vingolo, 1994). Our results indicate that the LR functions that were generated using the DWT descriptors that quantified ERG components concomitant with the b-wave (i.e., 20b and 40b) presented with PH-like shapes that complemented the traditional TD measurement of the b-wave (TD b-wave curve in Figure 3E). A similar match was also found between the DWT and TD descriptors of the a-wave. The fact that we were able to mimic the characteristic PH, a signature trait of the cone ERG LR function (traditionally generated from TD measures), using DWT descriptors suggests that selected DWT descriptors appraised physiological attributes of the cone ERG that covaried (see Pearson coefficients in Figure 3) with those obtained with TD measures.

As illustrated in Figure 8, there were also some important differences between the various DWT LR functions, such as the intensity of stimulation at which the maximal values were reached. DWT LR functions disclosed two maximal peaks, one at 0.39 log cd.s.m⁻² (maximal values for 40b, 80ops, 160ops, and Σ Energy; Peak 1 in Figure 8) and another one at 0.64 log cd.s.m⁻² (maximal values for 20b, Hölder exponent and Δ -variance; Peak 2 in Figure 8). In a previous study, we also showed that the flash intensity required to reach the maximal b-wave amplitude and to optimally develop the photopic OP response

(i.e., where OP2, OP3, and OP4 are fully developed) was (approximately) 0.3 and 0.6 log cd.s.m⁻², respectively (Lachapelle et al., 2001). The brighter intensity also generated the most complete response (in terms of number of detectable ERG components), a claim in accord with our finding that the Hölder exponent (descriptor of roughness or complexity of a waveform) also peaks at the brighter flash intensity (0.64 log cd.s.m⁻²). Moreover, as it can be seen in Figure 8, while some DWT descriptors (40b, 80ops, 160ops, and Σ Energy) reached a plateau with the brightest stimuli (from 1.63 to 2.23 log cd.s.m⁻²), others either continued to decrease (Hölder and Δ -variance) or slightly increased (20b).

DWT analysis of the ERG also allowed us to further dissect the a- and b-waves into separate subcomponents, each oscillating at a specific frequency band centered at 20 and 40 Hz which, from the results shown in Figure 3A and B, appears to be distinct from each other. The latter indicates that the intensity of the stimulus significantly modulates the timefrequency composition of the ERG and consequently its morphology as evidenced with the different ERG waveforms shown in Figure 2A. Knowledge of the latter not only provides us with a more refined approach to ERG shape quantification, but also suggests that some retinopathies could, for example, preferentially affect one frequency band (or DWT descriptor) more than the others, thus permitting a more precise ERG-based segregation of different retinal disease processes. The latter was demonstrated in Figure 7 (and accompanying Table 2) where patients affected with ON- and OFF-pathway anomaly had a specific attenuation of the 20b and 40b descriptors, respectively. Analogous findings were also reported by Barraco et al. (2011), who used the CWT to compare the scotopic awave of normal and achromate subjects. They showed that the scotopic a-wave of normal subjects was composed of three frequency components (20, 140, and 180 Hz) and that the higher frequency component was severely reduced in achromates, further suggesting that some retinopathies can preferentially affect one frequency band more than the others (Barraco et al., 2011).

The DWT also offered an alternative approach to assess the OPs without having to filter the broadband ERG signal. As shown with the scalograms of Figures 2B and 5A, OPs descriptors were computed as the average of five coefficients and, as a result, did include some low-energy (blue) coefficients, which contributed no more than those found outside

the selected (white) boundaries. Of interest, despite this more inclusive approach, we nonetheless obtained very high correlations (r = 0.9831) between TD and DWT OPs measurements (see Figure 3F). Obviously, we might have obtained better fits by selecting which coefficients to include on a case-by-case basis (cherry-picking approach), a strategy that would have required some data interpretation from the user or more complex interventions from the algorithm, all of which could potentially lead to undesired artifacts, subjectivity and variability. Although our OPs descriptors (80ops and 160ops) do not, unlike band-pass filtering, permit the analysis of the photopic OPs individually, our results (see Figure 3C) did show that the two OP frequency bands differed in their respective energy level, suggesting that they might monitor different features of the high-frequency components of the ERG. Of interest, another wavelet study (Forte et al., 2008) previously revealed that rat OPs also comprised two frequency components (i.e., 70-80 Hz and 120-130 Hz). This study also showed that the two OP components had distinct LR functions, suggesting that they might be evoked by different retinal sources. Similarly, Zhou, Rangaswamy, Ktonas, and Frishman (2007) reported that the photopic OPs of primates also contained two distinct frequency bands, namely the slow OPs (presumably generated by the amacrine cells) oscillating at 80 Hz and the faster OPs (presumably generated by the ganglion cells) oscillating at 150 Hz (Zhou et al., 2007). Like our study, the slower OPs were of the highest energy level. In our study, the LR functions of the 80ops and 160ops DWT descriptors followed a PH-like function, suggesting an intimate tie linking the genesis of the b-wave with that of the OPs, a claim previously advanced using TD measures (Guite & Lachapelle, 1990; Lachapelle, 1987, 1990; Lachapelle & Benoit, 1994; Lachapelle & Molotchnikoff, 1986).

Although both OPs descriptors (80ops and 160ops) were found to be significantly reduced (compared to control) in both CSNB and CPCPA patients (see Table 2), we did not find any significant difference between the 80ops or 160ops descriptors of the two patient groups. This contrasts with a study that was previously published by us in which we showed that these diseases were separable based on OPs measurements as patients affected with CSNB more specifically lose early OPs (OP2 and OP3), while patients affected with CPCPA preferentially lose the later OP (OP4; Lachapelle et al., 1998). However, in this previous study (Lachapelle et al., 1998) we looked at the amplitude of

OPs individually, while herein, we measured the overall OPs energy (average of five coefficients; see Table 1). This is not to say that it is impossible to look at early or late OPs individually with the DWT. Indeed, there are almost an infinite number of descriptors that one can calculate from the DWT scalogram, and individual assessments of the early and late coefficients of the 80ops or 160ops descriptors (instead of averaging the five coefficients) could have been used to quantify the energy level of early and late OPs. Alternatively, the ratio between the 160ops and 80ops descriptors could have been used to detect differences between CSNB and CPCPA. Supportive of the latter, in CSNB and CPCPA patients, the value of the 160ops-to-80ops ratio (obtained by dividing 160ops by 80ops) is of 0.49 ± 0.09 and 0.72 ± 0.11 , respectively—values that are significantly different (p < 0.00001) from each other. This result indicates that the low- (80 Hz) and high-frequency (160 Hz) OP bands can be differently affected by a given disease process and suggest a potential association of the low- (80 Hz; i.e., 80ops descriptor) and highfrequency (160 Hz; i.e., 160ops descriptor) OP descriptors to the OFF- and ON-pathway, respectively. In a recent study, Dimopoulos et al. (2014) reported that the light-adapted OPs were separated into two frequency bands and suggested the possibility of ON and OFF system representation. Data presented herein would support this claim.

The Hölder exponent (a measure of waveform complexity) also varied significantly as a function of the intensity of the stimulus (Figure 4). Although signal complexity remains somewhat of an abstract concept, the Hölder exponent can be defined as an ERG descriptor that accounts for the number of distinct elementary components (or frequency bands) that compose the signal, as well as the local irregularities that these components add to the waveform when they combine together. The notion of complexity is thus related to signal morphology because signals that do not have the same components will necessarily be of distinct shapes. For instance, in the examples shown in Figure 1C, the Hölder exponent increases from 0.53 (20 Hz ERG) to a maximum of 1.2 (for the 20 + 40 + 80 +160 Hz ERG). As can be seen in Figure 8, in the initial portion of the PH, the Hölder exponent (green curve) increases at a faster rate than the TD and DWT parameters, while all three parameters appear to be more similarly modulated in response to the brightest intensities. This was also seen with the lower amplitude ERGs (compare Figure 5D, E). However, since the Hölder exponent is amplitude-independent, we did not expect that it
would follow the traditional PH shape. That is, of course, unless we postulate that the ERG waveform would become progressively more complex as its amplitude increases. Consequently, we believe that the faster increase of the Hölder exponent observed in the initial rising phase of the PH simply confirms previous reports that claimed that the photopic ERG signal was a composite potential where brighter stimuli added new components (i.e., thus increasing waveform complexity) to the threshold ERG waveform (Berson, Gouras, & Hoff, 1969; Lachapelle & Molotchnikoff, 1986). Of all the ERG descriptors (TD or DWT) considered in this study, the Hölder exponent was that which was shown to be the least variable (CV = 4.14% [Hölder] compared to 17.25% [b-wave amplitude]) for the ERG at 0.64 log cd.s.m⁻², suggesting that it could allow us not only to detect subtle pathological changes at disease onset, but also to facilitate the monitoring (and severity grading) of changing ERG morphologies as the disease progresses towards nearly extinguished responses. This claim is best supported with the gradual decline in the value of the Hölder exponent seen with progressively smaller ERGs in normal subjects (Figure 5E) as well as with the significantly attenuated values obtained from pathological responses (Figure 7; Table 2).

Of interest, when analyzed with the DWT descriptors, both patient groups (CSNB and CPCPA) presented in Figure 7 and Table 2 had a unique DWT scalogram signature and were therefore statistically separable from each other using five of the eight DWT descriptors (values in italic in Table 2), but were not using TD descriptors. The latter indicates that DWT descriptors of the ERG can complement the diagnostic information obtained using TD descriptors. The DWT descriptors were also more affected (up to 8 SDs below the mean) than the traditional TD parameters (maximum of 2.5 SDs below the mean). The latter would suggest that DWT descriptors could also potentially detect subtle ERG anomalies even when the TD measures (amplitudes and timing) are still normal. Previous studies conducted on more specialized types of ERG signals further support the latter claim. For example, Miguel-Jimenez et al. (2010) used the DWT decomposition to reconstruct mfERG waveforms into various frequency bands and demonstrated the higher sensitivity of this approach to detect changes in glaucoma patients, compared to classical Humphrey visual field tests. Similarly, Rogala and Brykalski (2005) previously showed that DWT coefficients were superior to traditional TD measures in segregating normal

from pathological PERG waveforms. Results presented in Figure 7 and Table 2 also indicate that certain disease can affect specific DWT descriptors. As shown, the photopic ERGs of CSNB patients suffered from a pronounced attenuation of the low-frequency b-wave component (20b) compared to the better preserved high-frequency b-wave component (40b), and conversely, CPCPA patients were characterized by opposite findings (40b specifically reduced compare to the better preserved 20b). Of interest, given that patients affected with CSNB and CPCPA have a specific anomaly of the ON and OFF retinal pathway (Bech-Hansen et al., 2000; Dryja et al., 2005; Garon et al., 2014; Gregg et al., 2007; Lachapelle et al., 1998; Langrova et al., 2002; Miyake et al., 1987; Pusch et al., 2000; Quigley et al., 1996) and that we found a specific attenuation of the 20b and 40b in CSNB and CPCPA, respectively, our results suggest that the 20b and 40b descriptors might therefore be more closely related to the ON (20b) and OFF (40b) cone pathway contribution to the ERG response, respectively.

Previous studies of the PH phenomenon, using a variety of approaches such as early/late OPs analysis (Rufiange et al., 2002), long-flash ERG (Kondo et al., 2000), mathematic models (Garon et al., 2014; Hamilton et al., 2007), etc., suggest that the initial rising phase of the PH is OFF-response-dominated while the descending phase/plateau is ON-response-dominated. The fact that we have found (see Figure 3) significantly higher values of the 40b (OFF) compared to 20b (ON) in the rising portion of the PH, and conversely, significantly higher values of the 20b (compared to 40b) in the descending portion and plateau of the PH, further supports that the 20b and 40b descriptors can be associated to the ON and OFF response, respectively. Furthermore, in another study conducted on monkeys, Ueno et al. (2004) used pharmacological blockages to isolate the ON and OFF components of the primate cone ERG and showed that the OFF-component was delayed with higher intensities. Of interest, we also found that the 40b component occurred at a delayed position with the six brightest intensities (see white arrows in Figure 2C). Based on the above, our findings indicated that the PH phenomenon of the human ERG effectively results from the combination of both ON- and OFF-pathway activity, the initial rising phase being characterized by a greater contribution of OFF-pathway (40b) energy and the descent phase and plateau being characterized by a greater contribution of ON-pathway (20b) energy. Based on the above, it is not surprising that the ERG response of maximal complexity (and of high amplitude; b-wave amplitude >100 μ V) was reached at the intensity of 0.64 log cd.s.m⁻², where both the ON (20b) and OFF (40b) pathway are equally (and maximally) contributing to the response.

Finally, although the DWT analysis of the photopic ERG did permit the detection of major differences between CPCPA and CSNB waveforms, it does not mean that the DWT is the only technique that is able to segregate the two signals. As aforementioned, OP measurements in the TD did allow us to distinguish the two retinal disorders (Lachapelle et al., 1998). Likewise, Fourier analysis (such as the FFT) could also have been used to distinguish the two abnormal ERG signals by determining the frequency of the fundamental component (see Supplementary Figure S1). However, one should keep in mind that the FFT suffers from significant drawbacks when compared to the DWT. For example, the absence of temporal resolution in the FFT prevents the a-wave and b-wave from being independently quantified (since the a- and b-waves oscillate at overlapping frequencies) and also precludes the detection of latency shifts of the ERG components (Gauvin et al., 2014). Of interest, in the current article, the DWT allowed us to detect significant differences between the a-waves of CSNB and CPCPA patients and detected delays in the 40b component of photopic ERGs evoked from CPCPA patients as well as in ERGs recorded from normal subjects in response to the brightest stimulus intensities. Indeed, both of these potential diagnostic features are inaccessible if frequency domain analysis of the ERG waveform is limited to the FFT.

8. Conclusions

This study represents the first attempt to study the luminance-dependence of ERG components extracted with the DWT and the first application of wavelet analysis to study the ERG recorded from patients affected with retinal diseases impairing the retinal ON and OFF pathways. We characterized the LR functions of novel DWT descriptors of the human photopic ERG and demonstrated the usefulness of these descriptors in quantifying normal and pathological ERGs signals beyond what can be accomplished using the classical TD measures (e.g., a- and b-wave amplitude). Our results also suggest the possibility that a

given retinopathy may more specifically impair one of the DWT descriptors, raising the possibility that alterations of one or more DWT descriptors (or combinations of) could be pathognomonic for a given disease process (as suggested from the data shown in Figure 7 and Table 2). The DWT thus offers an alternative approach to identify (early diagnosis), classify, and possibly stage the different pathophysiological processes that impair retinal function-information that will be highly relevant, especially when assessing diagnostically challenging cases where the retinal disorder has not yet impaired TD measures of the ERG. Of interest, while delays in the ERG response (i.e., a TD measure) remain a critical descriptor of pathological ERGs, as shown in Figure 7, significantly delayed ERG b-waves can also retard the temporal position of some b-wave descriptors (such as that of the 40b descriptor in the CPCPA ERGs shown in Figure 7A), demonstrating that latency shifts can also be detected (and thus measured) with the DWT scalogram. Finally, although our data strongly support the fact that ERG analysis is more complete in the time-frequency domain, this is not to say that analysis of the ERG in the TD should be abandoned. Rather we advocate the use of the DWT approach as a complement to TD measures of the ERG.

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11. Captions to Figures and Tables

Table 1: Definition of the local wavelet maxima (LWM) descriptors. Notes: Column 1: The name by which the LWM is referred to in the text. Column 2: Time window within which the descriptor is localized. Column 3: The frequency band of the LWM. Column 4: How the descriptor is computed. Note that for 160ops, the first maximal coefficient is computed by taking the maximal value of the first two coefficients, the second maximal value is computed by taking the maximal value of the third and fourth coefficients, and so on until the fifth maximal value, which is computed by taking the maximal value of the last two coefficients. Column 5: Extent of the translations of the electroretinogram (ERG) that is used to optimally localize each LWM descriptor. Negative and positive values indicate a translation of the ERG to the right and to the left, respectively. Translations are achieved with increments of 1 ms.

Table 2: Analysis of ERGs obtained from congenital stationary night blindness (CSNB), congenital postreceptoral cone pathway anomaly (CPCPA), and control groups. Notes: Column 1: Names of the descriptors and parameters. Column 2: Values ($M \pm SD$) obtained in CPCPA group. Column 3: Values ($M \pm SD$) obtained in CSNB group. Column 4: Percent difference (computed as [(maximal value – minimal value) / minimal value] × 100) between the two patient groups. Column 5: Values ($M \pm SD$) obtained in control group. Bold: Value that is significantly different from control; Italic: Significant differences between CPCPA and CSNB; Bold + Italic: significant differences between CPCPA, CSNB, and Control. Values between square brackets (columns 2 and 3) indicate the number of standard deviations below or above the control mean (5th column) for that parameter, thus indicating the descriptions that were the most/least affected compared to control (highest value = most affected; lowest value = least affected). Values between parentheses (columns 5) indicate the coefficient of variation.

Figure 1: Identification of selected DWT descriptors. (A) DWT scalogram of a normal photopic ERG (B) evoked to a 0.64 log cd.s.m⁻² stimulus. The most prominent oscillatory component of the ERG appears as the darkest red rectangle (highest energy; see the calibration color bar) in the region of the scalogram where it is located (here centered at 40

Hz [frequency] and between 17.5 and 36.25 ms [time]), while the absence of oscillating components, at any given location, appears as the darkest blue rectangle (no energy). Almost an infinite number of descriptors can be calculated from the DWT scalogram. For example, the six LWM descriptors included in this study are identified (20a, 40a, 20b, 40b, 80ops, and 160ops) and delimited by white borders at (A). As shown, some LWM descriptors include a single wavelet coefficient (i.e., rectangle) within their borders, such as 20a, 40a and 20b, while others comprise several coefficients, such as 40b (two coefficients), 80ops (five coefficients), or 160ops (10 coefficients). For quantification purposes, LWM descriptors that included more than one coefficient were defined as the maximal or averaged value of included coefficients (see Table 1 for details). (B) Normal photopic ERG evoked to a 0.64 log cd.s.m⁻² stimulus. "a" and "b" identify the a- and bwaves and "OPs" identify two oscillatory potentials (OP2 and OP3). (C) The original ERG waveform (blue curves) is accurately (Pearson's score = 0.9853) reconstructed (i.e., inverse DWT; black curves) by summating the four DWT levels that included our LWM descriptors (20, 40, 80, and 160 Hz). (D-E) Wavelet variance analyses also allows for the calculation of robust DWT descriptors. For example, the blue curve of (D) represents the variance of the wavelet coefficients (i.e., expressed as the standard deviation) measured at each of the eight DWT levels (identified as Levels 1 to 8 in the DWT scalogram of [A]) and the red line represents the linear fit (R2 = 0.93) between Levels 2 and 5. The difference between the variance measured at Level 6 (50.13) and its prediction from the linear fitting (17.6) defines the Δ -variance descriptor (32.53 in this example). Furthermore, the natural logarithm of the wavelet variance data points shown in (E) was fitted by a linear model between Levels 2 and 6 (as represented by the red line; R2 = 0.91). The Hölder exponent (1.19 in this example) is defined as the slope of this linear fitting.

Figure 2: Representative ERG responses and associated DWT scalograms. (A) Composite ERG responses (i.e., obtained from arithmetic average of ERG responses obtained from 15 normal subjects) evoked to each of the 14 progressively brighter stimuli. "a" and "b" identify the a- and b-waves of the ERG and the vertical arrow indicates stimulus onset. (B) Luminance-response function of the b-wave amplitude obtained from the ERGs shown in (A). The black vertical line identifies the stimulus intensity (0.39 log cd.s.m⁻²), which

yielded maximal b-wave amplitude. (C) DWT scalograms of the 14 ERGs shown in (A). The LWM descriptors considered in this study (20a, 40a, 20b, 40b, 80op, 160ops) are identified in the 0.39 scalogram where they are delimited with white borders. For each scalogram, the color-coding was normalized to the LWM of maximal energy (see color bar on the right-hand side of the bottom-right scalogram). The frequency bands are indicated on the right-hand side of the top-right scalogram. White arrows indicate delayed positions of the 40b descriptors compared to that of the previous stimulus intensities. (D) Luminance-response function of the total energy included within the white borders (i.e., Σ Energy: 20a + 40a + 20b + 40b + 80ops + 160ops). The black vertical line identifies the stimulus intensity (0.39 log cd.s.m⁻²), which yielded the DWT with maximal energy content.

Figure 3: Luminance-dependence of LWM descriptors. (A–B) Mean (\pm 1 SD; only one direction shown for clarity) LR functions obtained using the LWM descriptors of the a-waves (i.e., 20a and 40a) and b-waves (i.e., 20b and 40b), respectively. Black asterisks located above and under the curves indicate when the 40 Hz descriptors are significantly (p < 0.05) higher or lower than those of the 20 Hz, respectively. (C) Mean (\pm 1 SD) LR functions obtained using the LWM descriptors of the OPs (i.e., 80ops and 160ops). The black asterisk in the middle of the black line located under the curves indicates that irrespective of the intensity, the 80 Hz OPs energy (80ops) was significantly larger (p < 0.05) than the 160 Hz (160ops). (D–E) The normalized summation of the mean 20-Hz and mean 40-Hz LR functions of the a- and b-wave obtained using the LWM (black curves) are superimposed on the normalized mean a- and b-wave amplitude LR functions measured using the TD approach (green curves). (F) The normalized summation of the mean 80-Hz and mean 160-Hz LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on the normalized LR functions (black curve) of the OPs is superimposed on

Figure 4: Luminance-dependence of WVA descriptors. (A) Mean (± 1 SD; only one direction shown) LR functions of the Hölder exponent. (B) Mean (± 1 SD) LR function of the Δ -variance descriptor. (C) Traditional LR function of the b-wave amplitude measured

in the TD. Black vertical lines indicate the stimulus intensity (0.39 or 0.64 log cd.s.m⁻²), which yielded the maximal value.

Figure 5: Analysis of low-amplitude ERGs. (A) Composite ERG responses (i.e., obtained from arithmetic average of ERGs obtained from 10 subjects) evoked to seven progressively dimmer intensities (in log cd.s.m⁻² given at the left of each tracings). The a- and b-waves are indicated as "a" and "b"; the vertical arrow (at top of first tracing) indicates stimulus onset. The horizontal and vertical calibration bars apply to each ERG, but for clarity, responses evoked at -1.81, -2.04 and -2.23 have been magnified 2, 4, and 8 times, respectively. Associated DWT scalograms in which the LWM descriptors have been surrounded by white borders are shown on the right-hand side of the waveforms. For each scalogram, the color-coding was normalized to the LWM of maximal energy. The frequency levels and the color bar are indicated on the top and bottom scalogram, respectively. (B) LR functions of the a- and b-waves' amplitude obtained from the ERG shown in (A). (C) Mean $(\pm 1 \text{ SD}; \text{ only one direction shown for clarity}) LR functions$ obtained using the DWT descriptors of the a-waves (i.e., 20a and 40a) and b-waves (i.e., 20b and 40b). (D) Mean (±1 SD; only one direction shown) LR functions obtained using the DWT descriptors of the OPs (i.e., 80ops and 160ops). (E–F) Mean (± 1 SD; only one direction shown) LR functions obtained using the Hölder exponent and Δ -variance, respectively.

Figure 6: Analysis of ERGs with low and high SNR. (A–B) Photopic ERGs recorded from normal subjects and evoked to stimuli of 0.64 or $-2.23 \log \text{cd.s.m}^{-2}$, respectively. The blue ERG (A1 and B1) shows the raw waveform and the superimposed red response (A1 and B1) represents the reconstructed ERG (i.e., obtained using the inverse DWT such as in Figure 1D). Finally, the black tracings shown below the ERG responses (A2 and B2) represent the baseline noise and residuals (i.e., obtained by subtracting the reconstructed ERG from the raw ERG). The noise amplitude (see vertical calibration bars) is about the same for both ERGs. In (A), the noise has a negligible effect (Raw ERG \approx Reconstructed ERG) on the ERG measurements (since the SNR is high). However, as shown in (B), the noise has a significant corrupting impact on the accuracy of the measurements (since the SNR is low). Thus, at low SNR, the noise contributes to the variance of the measurements. (C) DWT (dashed lines: a-wave [20a + 40a] and b-wave [20b + 40b] energy) and TD (full lines; a- and b-wave amplitude) variability (expressed as the CV) of a-wave (blue curves) and b-wave (red curves) measured in ERG responses evoked at two different stimulus intensities (-2.23 and 0.64 log cd.s.m⁻²).

Figure 7: Analysis of ERGs from patients affected with ON- and OFF-cone pathway anomalies. (A) A representative normal ERG response (Tracing 1) evoked to a stimulus of $0.64 \log cd.s.m^{-2}$ is compared to representative pathological ERGs (also evoked at 0.64 log cd.s.m⁻²) obtained from patients affected with an ON-specific (CSNB: Tracings 2 [composite response obtained from the average of all patients], 3, and 4 [representative responses obtained in two different CSNB patients]), or an OFF-specific (CPCPA: Tracings 5 [composite response obtained from the average of all CPCPA patients], 6, and 7 [representative responses obtained in two different patients]) cone pathway anomaly. The a- and b-waves are indicated as "a" and "b." The black arrow (at top of Tracing 1) indicates stimulus onset. The horizontal and vertical calibration bars apply to each ERG. Associated DWT scalograms in which the LWM descriptors have been surrounded by white borders are shown on the right-hand side of the waveforms. For each scalogram, the color-coding was normalized to the LWM of maximal energy. The frequency bands and the color bar are indicated on the top and bottom scalogram, respectively. White arrows indicate a preferred reduction of the 20b (scalograms of CSNB patients) or of the 40b (scalograms of CPCPA patients; note the delayed positon of the 40b in all cases) descriptors. (B–C) Group data showing the values (M and SD) of the 20b (B) and 40b (C) descriptors obtained in control subjects (black bars) and in CSNB (blue bars) and CPCPA (red bars) patients. (D) Values of the 40b-to-20b ratio obtained in control, CSNB, and CPCPA. Dashed line indicated a unitary ratio (i.e., 40b-to-20b = 1). Significant differences are indicated on each bar graph.

Figure 8: Normalized (between 0% and 100%) LR functions derived from the DWT descriptors that presented with various hill-like shapes in the present study (see figure legend). The black curve (identified as TD b) shows the traditional LR function of the b-wave amplitude measured in the TD. The solid vertical lines (lines 1 and 2) indicate the

0.39 and 0.64 log cd.s.m⁻² stimulus intensities that evoked the maximal values (Peak 1 and Peak 2) of the descriptors. As seen in the insert, four DWT descriptors reached their maximal value at Peak 1 and three descriptors at Peak 2.

Figure S1: Fourier analysis of the averaged (mean of all patients) CSNB (top panel) and CPCPA (bottom panel) ERGs. The frequency spectrums are shown as amplitude spectrums. The gray arrows indicate the frequency of the maximal component that contributes to these ERGs (CSNB: 33.33 Hz; CPCPA: 20 Hz).

Figure S2: (A and B) 20a and 40a values obtained while shifting the 0.64 log cd.s.m⁻² ERG waveform to a given extent (20a: -8 to 8 ms; 40a: -8 to 4 ms; as reported in Table 1). Negative and positive translation values shift the ERG to the right and left direction, respectively. Values obtained without translation (i.e. Translation = 0 ms) are indicated with the blue circles (20a: 67.06; 40a: 86.64) while maximal values obtained with translation are indicated with the red circles (20a: 75.24; 40a: 107.2). (C and D) 20a and 40a values obtained while shifting the 0.64 log cd.s.m⁻² ERG waveform to a greater extent to the left direction (20a: -8 to 16 ms; 40a: -8 to 8 ms). Overshifting the ERG to the left optimally align the b-wave onset under the 20a/40a boxes and should be avoided to prevent b-wave contamination of the a-wave measurement (sections highlighted in gray).

Figure S3: (A and B) Mean (\pm 1 SD) luminance-response functions obtained by processing the DWT descriptors of the a-wave (20a + 40a) and b-wave (20b + 40b) without translation or with translations. Although the energy is considerably underestimated when assessed without translation (blue curves), the obtained luminance-dependence patterns nonetheless correlate (a-wave: r=0.9795; b-wave: r=0.9598) with those obtained with translations (red curves).

12. Appendix A: Methodological details

12.1 Calculation of the DWT

In order to localize the energy content of the ERG in both time and frequency we computed the DWT of each ERG as follows:

$$DWT(j,k) = \int_{-\infty}^{\infty} x(t) 2^{-j/2} \overline{\Psi(2^{-j}t-k)} dt$$

DWT(j, k) represents the wavelet coefficients localized at discrete scales (indexed with j and corresponding frequencies in $[(Fs / 2)2^{-j}, (Fs / 2)2^{-j+1}]$, where Fs is the sampling frequency) and discrete time k, x(t) designates the raw ERG time series, and $\overline{\psi}$ denotes the complex conjugate of the mother wavelet. The DWTs were computed using the fast wavelet transform algorithm of Mallat (2009) implemented with MATLAB and Wavelab 850 routines (Buckheit, Shaobing, Donoho, Johnstone, & Scargle, 2005). Prior to DWT computation, each ERG was padded with 256 constant samples (by repeating the first and last value of the signal) on both sides of the response, in order to reduce edge effects (Torrence & Compo, 1998). With our parameters (i.e., 1,024 samples per padded signal and Fs of 3413.33 Hz), we were able to obtain 10 levels of decomposition per DWT. Only the first eight levels were displayed and the padding was discarded after computation (obtaining an eight-level time-frequency plan of 150 ms in length). Finally, several mother wavelets could have been used to extract the DWT descriptors described herein. However, one should be aware that use of different wavelets may affect the output of the DWT (such as the energy level), and hence, the same mother wavelet should be applied to normal subjects and patients for clinical decisions. In this study, we opted for the Haar wavelet to analyze the LWM descriptors of the ERG and for the symmetric Daubechies wavelet (set with two vanishing moments) to compute the WVA descriptors. Rationale for the use of these mother wavelets is indicated in the Methods section (see subsection "Selection of mother wavelets").

12.2 DWT Quantification of the ERG waveform with LWM descriptors

Six LWM (illustrated in Figure 1A and defined in Table 1) were considered in this study, namely 20a, 20b, 40a, 40b, 80ops, and 160ops. Furthermore, given that translation

of the ERG response is expected (such as peak-time variation of ERGs) and that the DWT is not a shift-invariant transform (Guo, 1995), we computed, as previously suggested (Coifman & Donoho, 1995), the DWT of several translated versions (translation of the entire ERG waveform) of the same ERG signal in order to optimize the identification of the maximal LWM independently of the timing (peak time) of the a-wave, b-wave, and OPs. These translations allow the various components of the ERG to be optimally aligned under their associated wavelet boxes (such as that of the a-wave under the 20a and 40a boxes), thus compensating for the lack of shift-invariance of the DWT. Each LWM was thus calculated as the local maximum value obtained while shifting the ERG waveform to a given extent. The width of these translations is reported in Table 1 (column 5) for each LWM descriptor. Each translation was conducted with increments of 1 ms, and negative and positive translation values indicate displacement of the ERG signal to the right and left, respectively. As indicated in Table 1, the translation extent of the a-wave descriptors (20a and 40a) does not allow as much translation to the left (i.e., positive translation values) compared to that allowed for the b-wave descriptors (20b and 40b). In limiting leftward translations, we prevent the alignment of the b-wave under wavelet boxes associated with the a-wave (20a and 40a) and therefore avoid the potential contamination of the b-wave on the 20a and 40a descriptors (see Supplementary Figure S2). One also notes (see Table 1) that the higher the frequency, the smaller is the magnitude of the translations. This is due to the fact that the higher the frequency is, the smaller the scale will be (i.e., width of the rectangular boxes in the scalogram), resulting in a higher temporal resolution of the DWT. Consequently, for smaller scales, minimal translations are needed to obtain optimal alignments. When the LWM descriptors are directly assessed without translation of the ERG responses, the resulting energy is considerably underestimated. This is better illustrated in Supplementary Figure S3, comparing the DWT LR function of the a- and bwaves obtained with (red curves) and without (blue curves) translations. Of note, although LR functions assessed without translations lead to an underestimation (i.e., inaccurate measure) of the energy, they are nonetheless reminiscent of those obtained with translations, in that they exhibit similar stimulus-dependent patterns.

12.3 DWT quantification of the ERG waveform with WVA

Wavelet variances (expressed as standard deviation) were computed using the values of 256 wavelet coefficients for the first DWT level (i.e., 1280-Hz band) down to two coefficients on the last level (Level 8; 10-Hz band). Note that in the DWT scalogram, the wavelet coefficients are seen as the colored rectangles of various sizes (see Figure 1A; the 256 smallest rectangles are found on the first level and the two largest on the eighth level). The eight standard deviation values thus obtained were then plotted against their respective DWT level (see example in Figure 1D). This graphic representation allowed us to define the Δ -variance descriptor, which, as illustrated in Figure 1D, was defined as the difference between the variance measured at Level 6 and its prediction at the same level based on the linear fit between Levels 2 to 5. The linear fitting was achieved between Levels 2 and 5 as this portion of the curve permitted an optimal linear fit, while Level 6 is where the curve stopped behaving linearly (see major discontinuity between Levels 5 and 6 in Figure 1D). The Hölder exponent is an amplitude-independent descriptor used to assess the regularity (often associated to the complexity) of a signal. The DWT offers an optimal scheme to estimate this exponent. This is due to the fact that the variance of the wavelet coefficients d at a given level j varies as $Var(d_i) \sim 2^{jH}$. Accordingly, the Hölder exponent H (also termed scaling exponent) can be estimated from the DWT variance-level plot, such as that illustrated in Figure 1D (Abry et al., 2002). Briefly, we process the natural logarithm of the variance-level curve (Figure 1D) to obtain the log-variance plot, such as the one shown in Figure 1E. This logarithm linearizes the curve (compare Figure 1D, E). The measurement of the Hölder exponent is then reduced to the calculation of the slope over the alignment region in the log-variance diagram. In this study, the linear fitting was achieved between Levels 2 to 6 (red line in Figure 1E) as this region of the curve offered the best linear fitting.

13. Figures and Tables

Table 1:

Descriptors	Time interval (ms)	Frequency (Hz)	Computing Details	Translations (ms)
20a	-20 to 17.5	$20 \pm 20/3$	Value of the coefficient	-8 to 8
20b	17.5 to 55	$20 \pm 20/3$	Value of the coefficient	-8 to 16
40a	0 to 17.5	$40 \pm 40/3$	Value of the coefficient	-8 to 4
40b	17.5 to 55	$40 \pm 40/3$	Maximal value of the 2 coefficients	-8 to 8
80ops	8.125 to 55	$80 \pm 80/3$	Mean value of the 5 coefficients	-4 to 4
160ops	8.125 to 55	$160 \pm 160/3$	Mean value of 5 maximal coefficients	-2 to 2

Table 2:

	CSNB $(n = 10)$	CPCPA $(n = 10)$	% Difference	Control $(n = 15)$
Anomaly	ON-Pathway	OFF-Pathway	n/a	None
Intensity	0.64	0.64	n/a	0.64
TD a	23.73 ± 7.45 [1.2]	$27.50 \pm 7.64 \ [0.6]$	15.89	31.11 ± 6.31 (20.3)
TD b	75.72 ± 21.05 [1.9]	63.86 ± 6.32 [2.5]	18.57	$112.77 \pm 19.45 (17.3)$
20a	$85.67 \pm 23.47 \ [0.5]$	$66.72 \pm 18.62 \ [0.7]$	28.40	77.08 ± 15.77 (20.5)
40a	69.63 ± 18.87 [1.7]	$99.82 \pm 16.98 \ [0.4]$	43.36	$107.96 \pm 23.22\;(21.5)$
20b	88.51 ± 30.22 [3.5]	203.06 ± 28.52 [1.0]	129.42	245.71 ± 44.69 (18.2)
40b	<i>174.16</i> ± <i>57.18</i> [2.2]	86.48 ± 9.46 [4.4]	101.39	256.45 ± 38.27 (14.9)
80ops	51.90 ± 16.71 [3.1]	41.58 ± 9.28 [3.8]	24.82	95.50 ± 14.20 (14.8)
160ops	25.23 ± 7.89 [3.4]	29.59 ± 5.45 [2.9]	17.24	54.43 ± 8.64 (15.9)
Hölder	0.85 ± 0.04 [6.6]	0.78 ± 0.04 [8.0]	8.97	1.18 ± 0.05 (4.1)
∆-Variance	22.45 ± 6.41 [1.0]	<i>14.42</i> ± <i>3.10</i> [2.3]	55.69	28.76 ± 6.3 (21.9)

Figure 1:



Figure 2:



Figure 3:



Figure 4:



Figure 5:



Figure 6:



Figure 7:



50 ms

Figure 8:



Figure S1:



Figure S2:



Figure S3:



1. Preface to chapter IV.

In chapter III (manuscript #2), accurate time-frequency descriptors of the a-wave, b-wave and OPs were identified, and their usefulness demonstrated in high- and very lowvoltage (< 1μ V) ERGs. The fact that the DWT was able to reproducibly assess lowamplitude ERGs suggest that the DWT should be of high diagnostic relevance, especially if one wishes to use the ERG to monitor disease progression in severe degenerative retinopathies (such as RP), whose final outcomes are often characterized by severely attenuated ERGs. Moreover, DWT descriptors were shown to be highly reproducible (e.g. Hölder Exponent CV = 4.14%), suggesting that the DWT could not only allow us to facilitate the monitoring of changing ERG morphologies as a disease progresses towards nearly extinguished responses, but also to detect subtle pathological changes at disease onset. While the DWT provided us with a more refined approach to ERG shape and ON and OFF pathway quantification, the claim that some degenerative retinopathies could, for example, preferentially affect one time-frequency component (or DWT descriptor) more than the others was not addressed. The latter would permit an early diagnosis and a more precise ERG-based segregation of different retinal disease processes or stages. The purpose of our third manuscript (Chapter IV) was thus to test the early-detection and better segregation claims in degenerative retinopathies such as RP. In order to do so, we first made use of a very peculiar case of highly asymmetrical RP. The inter-ocular difference in the pathological sequence of events of this unique patient allowed us to witness the earliest manifestation of the RP disease process (to test early DWT detection against TD) and also to see how it progresses to yield the full end-stage picture of RP. This patient confirmed our claim that the DWT descriptors could more rapidly detect subtle, and statistically significant changes at disease onset. Finally, we also made use of a cohort of 20 RP patients presenting with ERGs of different morphology to investigate if their ERGs could be further segregated with the DWT irrespective of patient' stages or etiology. Our results revealed 3 patterns; 1-preferred 40 Hz anomaly, 2-preferred 20 Hz anomaly, 3- uniformly distributed 20 and 40 Hz anomalies. The above would suggest that in some instances, the RP disease process would more severely impair the OFF or ON retinal pathway or have no preferential effect to either retinal pathway.

2. Manuscript Title.

CHAPTER IV

Manuscript 3 (Published Case Study Article)

WITNESSING THE FIRST SIGN OF RETINITIS PIGMENTOSA ONSET IN THE ALLEGEDLY NORMAL EYE OF A CASE OF UNILATERAL RP: A 30 YEAR FOLLOW-UP

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3. Abstract

Purpose. A patient initially presented with constricted visual field, attenuated retinal vasculature, pigmentary clumping and reduced ERG in OS only, suggestive of unilateral retinitis pigmentosa (RP). This patient was subsequently seen on 8 occasions (over 3 decades) and, with time, the initially normal eye (OD) gradually showed signs of RP-like degeneration. The purpose of this study was to evaluate which clinical modality (visual field, funduscopy or electroretinography) could have first predicted this fate. Methods. At each time points, data obtained from our patient was compared to normative data using Ztests. **Results**. At initial visit, all tests were significantly (p<0.05) altered in OS and normal in OD. Visual field and retinal vessel diameter in OD reduced gradually to reach statistical significance at the 5th and 6th visit (21 and 22 years after the first examination, respectively). In OD, the amplitude of the scotopic and photopic ERGs reduced gradually and was significantly smaller than normal at the 2nd (after 11 years) and 3rd visit (after 18 years), respectively. When the photopic ERG was analyzed using the discrete wavelet transform (DWT), we were able to detect a significant change at the 2nd visit (after 11 years) instead of the 3rd visit (18 years). Conclusions. Our study allowed us to witness the earliest manifestation of an RP disease process. The ERG was the first test to detect significant RP changes. A significantly earlier detection of ERG anomalies was obtained when the DWT was used, demonstrating its advantage for early detection of ERG changes.

4. Introduction

Retinitis pigmentosa (RP), a progressive retinal disorder, is usually characterized with the following clinical signs: nyctalopia and visual field (VF) constriction (to tunnel vision) [1–3], progressive narrowing of the retinal blood vessels, bone spicule-like pigmentary deposits and pallor of the optic nerve head [4–6], a gradual reduction in amplitude of the rod (first affected) and, with progression, of the cone-mediated electroretinograms (ERGs) [7, 8] and finally, blindness in the most severe cases. The above clinical signs are usually present in both eyes albeit not necessarily to the same extent.

Although it is well known that RP is a progressive disorder, little is known on how it progresses with time. Grover et al. [3] measured the VF area as a function of the age of RP patients in order to model the average yearly rate of VF loss and found that the averaged half-life of the VF values was 7.3 years. Similarly, Holopigian et al. [9] modeled the rate of deterioration of visual acuity (VA), Goldmann VF and focal ERG amplitude over 9 years in patients affected with RP. They concluded that measures of peripheral vision (i.e. Goldmann VF) degenerated faster than those assessing central vision (VA and focal ERG) and that rates of decline measured with the different tests varied significantly among patients. More recently, it was demonstrated, in a large population study of RP patients, that the degeneration pattern, as determined with the 30 Hz cone ERG, could be modeled using an exponential curve, with a fast degeneration at the onset and a progressively slower rate of change as the patient aged [10]. The above-mentioned studies greatly helped in understanding how RP progresses with time. However, given that these studies were conducted on patients that already had signs of the RP degenerative process (such as nyctalopia, fundus anomalies, VF constriction or ERG attenuation) at initial visit, it prevented us from identifying the earliest sign of the RP degenerative process. In fact, to the best of our knowledge, no study has yet reported a structurally and functionally normal human retina that progressively degenerated to end with a complete RP picture.

In this report, we present findings obtained from a patient that initially presented with typical RP-like features in the left eye (oculus sinister, OS) and normal fundus, visual field and electroretinogram in the right eye (oculus dexter, OD), suggesting the diagnosis of unilateral RP (URP). This patient was subsequently seen on eight occasions over a time
span of nearly 30 years, and, with time, the allegedly normal eye gradually developed clinical signs suggestive of a RP disease process. The purpose of this study was to evaluate which clinical test (visual acuity, visual field, retinal fundus or electroretinography) could have first predicted this fate and to report, for the first time, an initially normal human retina that progressively degenerated to end with a complete RP picture.

5. Materials and Methods

5.1 Patient

A 31-year-old woman initially presented (in 1984) with decreased visual acuity in her left eye (OS) and complaints of night vision difficulties. Clinical examination of OS revealed a constricted visual field, pigmentary clumping and significantly attenuated scotopic and photopic ERGs. In contrast, results obtained from the right eye (OD) were all within the normal limits, supportive of the diagnosis of URP. Serology and family history were negative. DNA analysis (performed in 2012) was also negative to known RP mutations. This patient was subsequently followed up (visual acuity, visual field, retinal funduscopy and electroretinography) over a time span of three decades for a total of eight examinations (1984, 1995, 2002, 2004, 2005, 2006, 2008 and 2012) in order to monitor the progression of the RP disease process. Cystoid macular edema in OU (OS > OD) and cataract (OS > OD) were also seen with time. This study was approved by the Institutional Review Board of the Montreal Children's Hospital.

5.2 Visual acuity

Visual acuities (VAs) were obtained from both eyes at eight time points (1984, 1995, 2002, 2004, 2005, 2006, 2008 and 2012) using Snellen charts. In order to report the VA progression over time, the VAs were expressed as decimal values (i.e. 20/20 = 1; 20/40 = 0.5). If a line was not completely read by the patient, the decimal ratio was adjusted according to a previously reported method [11]. When appropriate, the light perception (LP) or no light perception (NLP) notations were also used.

5.3 Visual Fields

Visual fields (VFs) were obtained from both eyes at seven time points (1984, 1995, 2002, 2004, 2005, 2006 and 2008) using a Goldmann perimeter (Marco, Jacksonville, FL, USA). Visual field plots obtained using the I4e and IV4e isopters were digitized at identical resolutions (CanoScan LIDE 110, Canon, Tokyo, Japan) and imported into MATLAB R2015a (Mathworks, Natick, Massachusetts, USA) for processing. Briefly, the VF was converted into binary images that had two possible pixel values, namely 0 (black pixels) and 1 (white pixels), respectively. The white pixels (i.e. seeing part of the VF) were then counted to obtain quantitative measurements of the VF area.

5.4 Funduscopy

Fundus pictures were taken from both eyes at five time points (1984, 1995, 2002, 2006 and 2012) using the same fundus camera (Kowa, Naka-ku, Nagoya, Japan) and magnification. Posterior pole images (35-mm Kodachrome slides, Kodak, Rochester, New York, USA) were digitized at identical resolution (CanoScan LIDE 110) and imported into MATLAB for processing. Briefly, the vasculature was segmented into binary black and white images where the white pixels represent the retinal vasculature (arteries and veins). These images were then filled in red (arteries) or blue (veins) and superimposed over the original fundus pictures in order to facilitate the visualization and analysis of the retinal vasculature. Diameter measurements (in μ m) of the arteries and veins were obtained from the following four vessels of the temporal retina: inferior and superior temporal veins (ITV and STV) and inferior and superior temporal arteries (ITA and STA). The selected blood vessels remained easily identifiable at all time points. An average of three diameter measurements per vessel was used as the blood vessel outcome measure.

5.5 Electroretinography

ERGs were recorded from both eyes at seven time points (1984, 1995, 2002, 2004, 2005, 2006 and 2008) using either a corneal contact lens (Lovac: Medical Workshop Inc., Groningen, Netherlands; tracings of 1984 and 1995) or a DTL fiber electrode (27/7 X-

Static silver coated conductive nylon yarn: Sauquoit Industries, Scranton, PA, USA; tracings of 2002–2008). The contact lens electrodes were filled with methylcellulose and placed on the anesthetized cornea (drops of 0.5 % proparacaine), while DTL electrodes were positioned deep into the inferior conjunctival bags (anesthesia was not required) and secured at the external and internal canthi of each eye with adhesive tape [12–14]. Reference and ground electrodes (Grass gold cup electrodes filled with Grass EC2 electrode cream) were pasted at the external canthi and forehead, respectively. The patient was then placed in front of a full-field stimulator (Ganzfeld) with maximally dilated pupils (1% tropicamide), and photopic ERGs were recorded using a bright flash of 0.64 log cd s m⁻² delivered against a rod-desensitizing background of 30 cd m⁻². Following a 20-min period of dark adaptation, scotopic ERGs (-1.21 or 0.17 log cd s m⁻² stimulus) were obtained. ERGs (150 ms recordings including 20 ms of pre-stimulus baseline, 20-100 responses per average) were recorded with a 1-1000 Hz bandwidth (6 dB of attenuation) and amplified 10,000 times using a Grass P511 preamplifier (Grass technologies, West Warwick, Rhode Island, USA; tracings of 1984 and 1995) or a UTAS-E-3000 system (LKC Technologies Inc., Gaithersburg, Maryland, USA; tracing of 2002–2008). Finally, ERGs recorded in 1984 and 1995 were normalized (i.e. multiplied by a factor of 0.5) in order to be comparable to the recordings obtained with the DTL electrode according to a study conducted by our group [15].

The amplitude of the a-wave (when present) was measured from the baseline to the most negative trough of the ERG, and its peak time was measured from the onset of the stimulus to this trough. The amplitude of the b-wave (when present) was measured from the trough of the a-wave to the most positive peak of the b-wave, and its peak time was measured from the flash onset to peak. Since these measurements are taken in the time domain (TD), we will refer to them as TD measurements.

5.6 Wavelet Analysis

We also computed the discrete wavelet transform (DWT) from the raw ERG signals of both eyes at seven time points (1984, 1995, 2002, 2004, 2005, 2006 and 2008) using the fast wavelet transform algorithm of Mallat implemented with MATLAB [16, 17]. The DWT produces scalograms that include time (X-axis), frequency (Y-axis) and energy (Z- axis) variables. In the scalogram, the most prominent frequency component of an ERG appears as the darkest red (high energy) rectangle in the region of the scalogram where it is located (i.e. time and frequency coordinates), and conversely, the absence of frequency components will appear as dark blue (no energy) rectangles. The scalogram allows the identification of ERG components, each defined by different time and frequency intervals, thus obtaining a more comprehensive analytical description of the ERG signals as previously demonstrated by us and other [18-22]. Two DWT descriptors that were previously identified by us [21, 22] were used to quantify the energy of the 20 Hz (20 Hz \pm 6.6 Hz) and 40 Hz (40 Hz \pm 13.3 Hz) a-wave components (20a and 40a) and another two were used to quantify the 20 Hz and 40 Hz b-wave components (20b and 40b). The timefrequency locations of these DWT descriptors are shown in Fig. 7a (top scalograms). Finally, another DWT descriptor (i.e. Hölder scaling exponent) that describes more global features of the signal, such as the complexity of its morphology, was also included in this study and computed according to a method previously used by us [22]. We limited DWT analyses to the photopic ERG signals as they were always obtained using the same stimulus intensity (0.64 log cd s m^{-2}).

5.7 Statistical analyses

Using the above-defined methods, descriptive statistics [mean and standard deviation (SD)] were obtained from normal subjects for each outcome measure (visual field area: N = 30; blood vessels caliber: N = 20; TD and DWT descriptors of the ERG: scotopic N = 25; photopic N = 80). Normal visual acuity was defined as 20/20. To investigate which and when descriptors were significantly (p < 0.05) reduced throughout the course of the disease process, Z tests [23] and associated p values were computed for each of the outcome measure values obtained at each visit. Linear interpolation was also used to estimate p values between two successive follow-up visits. In contrast, we used the VA of the initially good eye at onset as the reference to evaluate when the VA acuity was diagnostically reduced (i.e. when the VA permanently became under the onset value). Finally, linear regressions were computed for each outcome measure over time, and ANOVAs were used to test the null hypothesis that the slopes (rate of change of the outcome measure value with time) were equal in both eyes.

6. Results

6.1 Visual acuity

The visual acuity obtained from each eye is illustrated in Fig. 1. As expected, despite some variation (nearly identical variation patterns in both eyes) in VA measures, we notice a progressive deterioration of the VA of both eyes with time. At the last visit, the VA of OS and OD was NLP and 0.2 (i.e. 20/80), respectively. Irrespective of the time point, the VA measured in OS was always lower than normal (i.e. 20/20) and was all lower than that of OD. The VA in OD became permanently smaller than that measured at initial visit at the 7th visit (after 24 years).

6.2 Visual field

Digitized VF plots of OS and OD are shown in Fig. 2a, b, and area variation with disease progression is graphically reported in Fig. 2c, d, respectively. As expected, at initial visit, the VF areas measured in OS were significantly smaller than those measured in OD (I4e: OS = 13.6 % of OD; IV4e: OS = 15.7 % of OD). Despite a slight increase in VF area measured at the 11-year follow-up (similar to VA measures), there was a progressive constriction of the VF in both eyes, to reach, at the last visit, I4e VFs of approximately equal area OU (OS = 8564 pixels; OD = 8261 pixels), corresponding to approximately 5 degrees of central vision. Overall (compare the red curves with the blue curves), both isopters showed similar patterns of visual field constriction. Irrespective of the time, VF area measured from OS was significantly (p < 0.05) reduced compared to normal values (as per Table 1) and was smaller (i.e. as much as 32 times smaller at the last visit with the IV4e isopter) than the one obtained from OD (except at the last visit with the I4e isopter). In contrast, VF areas measured in OD were within the normal range at the first four follow-up examinations (i.e. first 20 years) and, following a rapid deterioration, became significantly (p < 0.05) constricted at the 5th visit (after 21 years), with both isopters.

6.3 Funduscopy

As shown in Fig. 3a, at initial visit (1984) the peripheral retina of OS presented with pigment clumping (which covered 360°) and thinning of peripheral blood vessel, two characteristic RP features that could not be evidenced in OD (Fig. 3b). The above contrast

with results obtained at the last visit (2012) where both eyes presented (see Fig. 3c, d) with thinner than normal blood vessel diameters and bone spicule-like pigment clumping (white arrows) suggestive of RP. As shown in Fig. 4a–e, there was a gradual thinning of the retinal blood vessels as the disease progressed in OD. This is better illustrated in Fig. 4f where we report diameter measurements for the four major retinal blood vessels identified with white arrows in Fig. 4a [i.e. the superior (S) and inferior (I) temporal (T) arteries (A) and veins (V): STA, STV, ITA and ITV, respectively]. The most important diameter change was observed with the STV, which was found to be at 26.1 % of its initial diameter following the 28-year monitoring period. This change was considered to be significant (p < 0.05; normal values are given in Table 1) at the 6th visit (i.e. 22 years following the first examination). Likewise, the other blood vessels (i.e. ITA, STA and ITV) were also found to be at 32.3, 28.1 and 42.8 % of their initial diameters at the last visit, respectively.

6.4 Scotopic retinal function

Scotopic ERGs recorded from each eye are shown in Fig. 5a, b. One notices that at the initial visit (1984), the amplitude of the scotopic b-wave recorded from OS was smaller than that measured in OD (OS = 13.3 % of OD, which was of normal amplitude; see Table 1) and remained at this amplitude until 2002. In contrast, the ERG recorded from OD showed a gradual reduction in b-wave amplitude over the same time span and was significantly (p < 0.05) smaller than normal from the 2nd visit (11 years following the initial visit). At the last ERG session (2008), the scotopic ERGs of both eyes were extinguished, suggesting a complete extinction of rod-mediated function in both eyes as seen in the accompanying graph (Fig. 5c). Finally, when measurable, the peak times of the scotopic b-waves of both eyes remained within the normal range irrespective of the time point.

6.5 Photopic retinal function

The photopic ERGs recorded from both eyes are illustrated in Fig. 6a, b. At initial visit (1984), the amplitude of the photopic b-wave of OS was smaller than that recorded from OD (OS = 25.8 % of OD, which was of normal amplitude as per Table 1). With time,

the ERG amplitude of OS and OD became progressively smaller (Fig. 6c, d), resulting in a smaller signal-to-noise ratio (SNR) that complicated the accurate measurement of the ERG (especially for the last recordings which were mostly contaminated by 60-Hz noise). At the initial recording session (1984), the amplitudes of the a- and b-waves measured from OS were already severely and significantly (p < 0.05) reduced compared to normal, following which, further reduction was documented. In contrast, the amplitude of the first two recordings obtained from OD (1984 and 1995) was within normal amplitude limits (for both the a- and b-waves), following which, significant (p < 0.05) amplitude attenuation was observed for the a- and b-waves at the 3rd visit (after 18 years). Lastly, the progressive increase in the photopic a- and b-wave peak times measured from both eyes is shown in Fig. 6e, f. The a- and b-wave peak times measured in OS and OD became significantly delayed at the 6th visit (after 22 years).

6.6 DWT analysis of the photopic ERGs

DWT scalograms computed from the photopic ERGs shown in Fig. 6a, b are illustrated in Fig. 7a, b, respectively. The four DWT descriptors considered in the present study (20a, 20b, 40a and 40b) show that, irrespective of the ERG analyzed (except the recordings of 2008), the maximal energy of the signal was always contained within a time–frequency region delimited by the four descriptors. The 20 Hz b-wave descriptor (20b) was the most prominent component in 10 out of 14 recordings (OD and OS combined), while the 40 Hz b-wave descriptor (40b) was the maximal DWT descriptor in the other recordings. Despite the poor SNR of the last recordings (2008), the 20b descriptor remained that with maximal energy within the 20 Hz band, suggesting the presence of residual cone b-waves in both eyes (e.g. see the wavelet-reconstructed 2008 residual ERG trace in the insect of Fig. 6b). Furthermore, the 20 and 40 Hz a-wave descriptors (20a and 40a) were always smaller (as per energy scale) than the 20 and 40 Hz b-wave descriptors (20b and 40b), indicating that irrespective of the time point or eye considered, the b-wave was always the major components of the ERGs (i.e. no electronegative ERG).

The progressive attenuation of the 20 and 40 Hz photopic a- and b-wave energy descriptors (i.e. 20a, 20b, 40a and 40b) is shown for both eyes in Fig. 8a–d, respectively. At the initial recording session, all DWT descriptors of the a- and b-waves from OS (Fig.

8a, c) were already severely and significantly (p < 0.05) reduced compared to control values (reported in Table 1) and kept on decreasing with disease progression. In contrast, at the initial visit, DWT descriptors from OD (Fig. 8b, d) were all within the normal range. Significant (p < 0.05) attenuation in energy content was first detected for both the a- and b-waves at the 3rd visit (after 18 years; parameters: 20a, 40a and 20b) and 2nd visit (after 11 years; parameter: 40b), respectively. Furthermore, one notices that the 20 Hz and 40 Hz components of the a- and b-waves revealed different decay patterns with disease progression. This is better exemplified when comparing the 20a and 40a decays of OS (Fig. 8a), or the 20b and 40b traces of OD (Fig. 8d). As shown in Fig. 8e, f, the gradual deterioration of the cone response caused a progressive reduction in the value of the Hölder exponent, a morphology-sensitive, but amplitude-independent, ERG descriptor derived from the DWT scalograms. The Hölder exponents measured from OS were always significantly (p < 0.05) reduced compared to normal (value reported in Table 1) and OD as well. In contrast, the Hölder exponent measured from the ERG of OD was initially normal and was significantly (p < 0.05) smaller at the 2nd visit (after 11 years), following which, it continued to decrease with disease progression. The gradually smaller Hölder exponent that we measured in the ERGs of OD and OS suggests a gradual reduction in the number of distinct elements (or frequency bands) that compose the photopic ERG signals, and consequently, a reduction in ERG wave complexity.

7. Discussion

In a recent report, Weller et al. presented a 30-year follow-up of a case of unilateral RP where the diseased eye showed clinical signs suggestive of RP, which worsened with time. After 30 years, there were still no signs of a RP-like process in the fellow eye [24]. The latter clinical presentation differs from the data that we reported herein where, although our patient did initially present with RP-like features in OS and normal fundus, visual field and electroretinogram in OD, during the following 30 years or so, the initially normal eye (i.e. OD) gradually developed clinical signs (fundus, visual field and ERGs) suggestive of an RP-like retinal degeneration. This inter-ocular difference in the pathological sequence of events allowed us, for the first time to our knowledge, not only

to witness the earliest manifestation of the RP disease process but also to see how it progresses to yield the full clinical picture of RP (i.e. bone spicules, constricted visual fields, extinguished rod ERG and nearly extinguished cone ERGs).

More than 20 parameters were derived from the different ophthalmologic tests performed on this patient. ERG descriptors were the first to detect (rod b-wave amplitude: 2nd visit, i.e. after 11 years; photopic b-wave amplitude: 3rd visit, i.e. after 18 years) signs of an ongoing retinal disease in the allegedly normal eye followed, at a later stage, by the visual field area (at the 5th visit, after 21 years), the retinal vasculature (at the 6th visit, after 22 years) and, finally, the visual acuity (at the 7th visit, after 24 years), respectively. These results are in accordance with current knowledge of the RP disease progression, which established that retinal function (as determined with the ERG) is usually impaired several years prior to the appearance of the other clinical signs of RP, such as night blindness, visual field constriction/scotoma, decreased visual acuity and thinning of the retinal blood vessels [25]. While RP patients usually exhibit delays of the rod ERG response [25, 26], significant changes of the rod b-wave peak time were not detected in the time period monitored in this study.

Cases of URP are rare, accounting between 0.02 and 5 % of all RP cases, depending on the RP population studied [27–33]. According to "Francois and Veriest" criteria, to confirm a diagnosis of URP, one should follow a patient for 5 years at least in order to rule out a delayed onset of RP in the allegedly normal eye [34]. Our results demonstrate that the Francois and Veriest criteria should be revised to suggest the monitoring of URP patients for more than 5 years in order to conservatively rule out a delayed onset of RP. Several causes are known to trigger unilateral/asymmetrical RP-like retinal degeneration, including syphilis (and other inflammatory/viral/bacterial diseases or infections), optic disk vasculitis, central retinal artery occlusion, the use of certain drugs (thioridazine, chloroquine, etc.), and even traumatic injuries [35–38]. These causes could have explained the atypically pronounced asymmetry seen in our patient, but they were all ruled out by the various serology tests and as there was no history of trauma. Furthermore, with the recent advances in genetic, many genes have been identified and associated with RP [39–41]. However, DNA analysis (performed in 2012) was negative to known RP mutations and there was no family history of RP.

7.1 Rate of change and disease course

The rates of change (expressed as the slope of the linear fitting obtained during the monitoring period) of the different parameters of both eyes are reported in Table 1 and reveal that most parameters decayed significantly (p < 0.05) faster in OD. Overall (i.e. average of all the rates of change assessed), the ERG parameters degenerated 4.53 times faster in OD than OS. The progressive retinal degeneration associated with RP is known to usually cause an exponential decay (where the risk of cell death remains more or less constant or decreases exponentially with age) in the ERG amplitude (with a loss that varies between 8.7 and 18.5 % per year) [8, 42] as well as in visual field area where the loss is slower (2.6–13.5% of annual lost) [3, 42, 43]. The large variation in the previously reported rates of function decline may be attributable to the disease stage, variation in the affected genes, environmental factors or a combination of the latter. Of note, however, the kinetic of function decay measured in our patient did not appear to be exponential, but rather pseudo-sigmoidal. A sigmoidal decline in cell number would suggest an increasing risk of cell death associated with cumulative damage [44]. With this cumulative damage hypothesis, the time of death of any photoreceptor is random and the rate of cell death increases over time. This concept of randomness could explain the high inter-ocular differences that we report herein.

7.2 Advantages of using a refined ERG analysis approach for diagnosis and monitoring:

Of interest, pathological changes were detected much earlier (at the 2nd visit, after 11 years, compared to the 3rd visit, after 18 years) when sophisticated mathematical tool (i.e. the DWT) was used to analyze the cone ERG. The latter is well supported with the data reported in Table 1, which reveals (along with a previous study of ours [22]) that the Hölder exponent is the least variable DWT descriptor (CV = 4.86 %) of the normal ERG and the first one to detect a significant change in the ERG of OD. Furthermore, the gradual decline in the value of the Hölder exponent that we measured in the progressively more altered ERG responses of the patient (Fig. 8e, f) also confirms our previous claim [22] that

this descriptor could facilitate the monitoring (and severity grading) of the ERG as disease progresses to nearly extinguished responses. Similarly, we have previously shown that DWT analyses of the photopic ERG identify two sub-components of the a- and b-waves oscillating at a specific frequency band centered at 20 (20a and 20b descriptors) and 40 Hz (40a and 40b descriptors), respectively [21, 22]. As shown in Fig. 8a, d, the 20 and 40 Hz components appear to be distinct from each other, given that they follow distinct decay patterns with time. As reported in Table 1, the 40 Hz component (40b) was the first (at the 2nd visit, after 11 years) energy descriptor to be significantly altered in OD (compared to the 3rd (after 18 years) and 6th visit after 22 years) for the traditional b-wave amplitude and peak time measurements, respectively. Likewise, in OS, the 40b descriptor was markedly more affected than 20b at all time points (except at the last visit where the enhanced noise level might have corrupted the 40b value). To investigate whether the more severe 40 Hz anomaly that we documented herein was a feature of the RP disease process, DWT analysis of photopic ERGs obtained from selected patients (N = 20) affected with RP (of unknown genotypes) was performed. As illustrated in Fig. 9a (along with representative scalograms and tracings at Fig. 9b), in normal subjects the 40b/20b ratio is close to unity. Thus, a ratio smaller than 1 will indicate a preferential 40b anomaly of the ERG (such as that presented throughout this article), while a ratio greater than 1 will point to a preferential 20b anomaly. Of interest, for our RP cohort, the average 40b/20b ratio was of 0.78 ± 0.24 , a value significantly lower (p < 0.05) than control values (1.05 ± 0.06), indicating an overall attenuation of the 40b component. However, while 12 RP patients (60 % of patients) presented with a marked attenuation of their 40 Hz component, only 2 (10 % of our cohort) presented with a preferential 20 Hz anomaly and the remaining 6 patients (30 % of patients) had a ratio value that was not significantly different from normal. In a previous study of ours, we suggested that the 20 Hz (20b) and 40 Hz (40b) descriptors of the photopic ERG b-wave were closely associated with the ON and OFF retinal pathways, respectively. The above would therefore suggests that in most instances (60 %), the RP disease process would more severely impair the OFF retinal pathway, compared to ON pathway anomaly (10 %) or no preferential effect to either retinal pathways (30 %), a finding that supports a previous observation of ours [45]. It remains to be determined whether the above-mentioned ON-type, OFF-type or ON-OFF-type ERG anomalies characterize three different classes of RP disease process or whether it characterizes three different stages of any given RP disease process. Results shown in the present study would support the former claim.

Finally, as reported in Table 1, linear interpolation was used to estimate the time point, between two successive follow-up visits, where the measured parameter became significantly (p = 0.05) different from normal. Based on this interpolated data, it took 19.1 and 20.2 years to detect the first sign of RP using the traditional clinical test (size of retinal blood vessels and visual fields, respectively) compared to 8.5 and 15.5 years for the rod and cone ERG, respectively. Interestingly, the significantly faster detection of photopic ERG changes was obtained after 6.9 years when the discrete wavelet transform was used (Hölder exponent parameter). Although this approach can only provide an estimate of when (in years) a given parameter will become significantly (p = 0.05) different from normal, the marked difference (more than 4 years for the Hölder exponent) noted between the empirical and interpolated values strongly suggests a close monitoring (i.e. 3- to 5-year intervals) of these patients in order to detect functional signs of degenerative processes as soon as they manifest.

8. Conclusion

In conclusion, this study demonstrates that, using the traditional clinical tests (fundus examination and visual fields), we had to wait until at least the 5th visit (after 21 years) to detect the first sign of RP compared to the 2nd (11 years) and 3rd visit (18 years) for the rod and cone ERG (amplitude and peak time measurements), respectively. A significantly faster detection of photopic ERG changes was obtained when sophisticated mathematical tool was used. Our results not only demonstrate the advantage of the DWT for early detection of significant alteration in retinal function, but also as a monitoring tool that can be used even when the residual ERGs (which are often contaminated by unwanted noise) bear little resemblance with the normal waveform. This finding should positively impact the managing (diagnosis, prognosis and monitoring) of patients. However, the inter-ocular differences in the rates of changes (4.5 times faster in OD than OS) that were

documented herein also unfortunately demonstrated how difficult it can be for a clinician to predict the fate (and when it will occur) of a retinal degeneration, as even in the same patient, the degeneration rate can be extremely asymmetrical. Clearly, additional long-term follow-ups of disease progression using refined quantitative methods of analyses are warranted to improve the diagnostic sensitivity and specificity and to more rapidly rule out unilateral conditions.

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11. Captions to Figures and Tables

Figure 1: Visual acuity (VA) deterioration of the patient measured from OS and OD. The VA is expressed as a ratio (i.e. 20/20 = 1; 20/40 = 0.5). VA of LP and NLP corresponds to light perception and no light perception, respectively. Time is indicated as time since the first follow-up (i.e. 1984 = 0). VAs measured in OS were all severely reduced, while the vertical dashed line indicates the interpolated time point after which the VA of OD became permanently lower than its initial value (i.e. after 22.3 years).

Figure 2: a, **b** Progressive constriction of the visual fields of the patient measured with I4e and IV4e isopters in OS and OD. Progression is shown from top to bottom and indicated as time since the first follow-up (i.e. 1984 = 0 year) or as the year of the test (red text). **c**, **d** Progressive visual field constriction (blue traces isopter I4e; red traces isopter IV4e) computed from OS and OD using the digitized Goldmann visual fields shown in **a**, **b**, respectively. Irrespective of the year and isopter, the visual field of OS was significantly (p < 0.05) reduced, while in OD, the vertical dashed line marked with an asterisk indicates the interpolated time point after which the visual field area first became permanently significantly (p < 0.05) reduced (i.e. after 20.2 years using the I4e isopter).

Figure 3: a, **b** Initial retinal funduscopy obtained at three different positions in OS and OD at the first visit in 1984. **c**, **d** Retinal funduscopy obtained in OS and OD at the last visit in 2012. The white arrows indicate the retinal area where pigment clumping could be observed over 360°.

Figure 4: a–**e** Progressive thinning of the retinal blood vessels seen in the retinal fundus pictures of the posterior pole region (optic disk = green circles) obtained from OD. Arteries (red vessels) and veins (blue vessels) have been put in emphasis using MATLAB. In Fig. 3a, the superior (s) and inferior (i) temporal (t) arteries (a) and veins (v) are indicated (white arrows) as STA, STV, ITA and ITV, respectively. The inferior, superior, nasal and temporal sides are identified at a by the letters I, S, N and T, respectively. The year of the tests and the time since the first follow-up visit are shown at the bottom and top left corners of each panel, respectively. The scale bar (**a** bottom right corner) applies to each fundus images. **f** Progressive thinning of the superior and inferior temporal arteries and veins measured from the fundus picture shown in **a**–**e**. The legend is associated with the arteries and veins indicated by the white arrows at **a**. Time is indicated as time since the first follow-up visit (i.e. 1984 = 0). The vertical dashed line marked with an asterisk indicates the interpolated time point at which one of the blood vessels of OD became significantly (p < 0.05) attenuated (i.e. STV, after 19.1 years).

Figure 5: a, b Scotopic ERGs recorded from OS and OD at three time points and evoked to white flashes at the intensity of $-1.21 \log \text{ cd s m}^{-2}$. The progression is shown from top to bottom and indicated as time since the first follow-up (i.e. 1984 = 0) on the right-hand side of each waveform, and the year of recording is specified on the left-hand side of the tracings. The vertical arrows correspond to the flash stimulus onset. Vertical and horizontal calibration bars are shown on the left-hand sides and at the bottoms of both panels, respectively. **c** Progressive scotopic b-wave amplitude reduction measured from OS and OD at seven time points between 1984 and 2008. Since two different stimulus intensities ($-1.21 \log \text{ cd s m}^{-2}$: ERGs of 1984, 2002, and 2008; 0.17 log cd s m⁻²: ERGs of 1995, 2004, 2005 and 2006) were used in that 24-year interval, we represented the amplitude as a percentage of control (i.e. amplitudes were expressed as the percentage of an average of 25 controls at the same stimulus intensity). Time is indicated as time since the first follow-up visit (i.e. 1984 = 0). Amplitudes measured in OS were all significantly altered, while in OD, the vertical dashed line marked with an asterisk indicates that from that interpolated time point (i.e. 8.5 years), the scotopic b-wave was significantly reduced (p < 0.05)

Figure 6: a, b Photopic ERGs recorded from OS and OD and evoked against a background luminance of 30 cd m-2 using white flashes at the intensity of 0.64 log cd s m⁻². The progression is shown from top to bottom and indicated as time since the first follow-up (i.e. 1984 = 0) on the right-hand side of each waveform, and the year of recording is specified on the left-hand side of the tracings. The vertical arrows correspond to the flash stimulus onset. Vertical calibration bars are shown on the left-hand side of both panels and horizontal calibration at the bottom of **b**. Attenuated waveforms (see insets delimited by dashed lines) obtained from OS (between 2004 and 2008) and OD (between 2005 and 2008) were further amplified (see calibration bars). A non-measurable waveform from 2008 is reconstructed (red curve at **b**) using the inverse wavelet transform (20 and 40 Hz frequency band) to illustrated the hidden response that is quantified by the wavelet approach. **c**, **d** Progressive attenuations of the photopic a- and b-wave amplitudes measured from OS and OD in the ERG shown in **a**, **b**. Time is indicated as time since the first follow-up visit (i.e. 1984 = 0). The a- and b-wave amplitudes measured in OS were all significantly altered, while in OD, the vertical dashed lines marked with asterisks indicate that from

these time points (i.e. 16.4 and 15.5 years, respectively), the photopic a- and b-wave amplitudes were significantly (p < 0.05) reduced, respectively. **e**, **f** Progressive increase in the photopic a- and b-wave peak times measured from OS and OD in the ERG shown in **a**, **b**. Time is indicated as time since the first follow-up visit (i.e. 1984 = 0). The a- and b-wave peak times measured in OS were significantly altered after 22 and 21.3 years, respectively, while in OD, the vertical dashed lines marked with asterisks illustrate that from these interpolated time point (i.e. 21.2 and 21.5 years), the a- and b-wave peak times were significantly (p < 0.05) delayed, respectively. Black interrogation marks (located at time = 24 years in **c**-**f**) symbolize amplitudes and peak times that were not measured due to undistinguishable a- or b-waves in the 2008 tracings of **a**, **b**.

Figure 7: a, **b** Discrete wavelet transform (DWT) scalograms computed from the photopic ERGs shown in Fig. 6a, b for OS and OD, respectively. The time progression is shown from top to bottom. The year of recording and the time since the first follow-up visit (i.e. 1984 = 0) are indicated at the middle top and top right of each scalogram, respectively. The frequency bands are indicated in Hertz (Hz) on the left-hand side of **a**. The time is indicated in milliseconds (ms) at the bottom of both panels. The flash stimulus onset corresponds to time = 0 ms. The energy level (μ V s) is indicated on the color bars located on the right-hand side of each scalogram (minimal and maximal energy corresponds to the bluest and reddest color, respectively). The time–frequency localization of four analyzed DWT descriptors is indicated as 20a, 20b, 40a and 40b on the top scalogram of **a** and associated energy is quantified for each scalogram in Fig. 8a–d.

Figure 8: a–d Progressive reduction in the photopic 20 and 40 Hz a- and b-wave energies (i.e. 20a, 20b, 40a and 40b descriptors identified in Fig. 7a) measured from OS and OD in the DWT scalograms shown in Fig. 7a, b. The summation of the descriptors (20a + 40a and of 20b + 40b) are also graphed (black traces) in their respective panels. Time is indicated as time since the first follow-up visit (i.e. 1984 = 0) at the bottom of panels **e** and **f**, respectively. The a-wave (20a and 40a) and b-wave (20b and 40b) energies measured in OS were all significantly altered, while in OD, the vertical dashed lines marked with asterisks indicate that from these time points [i.e. 13.5 (40a) and 9.9 years (40b),

respectively], at least one descriptor of the photopic a- and b-wave energies was significantly (p < 0.05) reduced, respectively. **e**, **f** Gradual reduction in the ERG complexity measured from OS and OD using the Hölder exponent at seven time points between 1984 and 2008 from the DWT scalograms shown in Fig. 7a, b. Hölder exponents measured in OS were all significantly altered, while in OD, the vertical dashed line marked with an asterisk indicates that from that interpolated time point (i.e. 6.9 years), the photopic ERG complexity was significantly reduced (p < 0.05).

Figure 9: Analyses of the 20b and 40b DWT components computed from the photopic ERGs (stimulus intensity: 0.64 log cd s m^{-2} ; background: 30 cd m^{-2}) obtained from 20 patients affected with retinitis pigmentosa (RP) of different stages and etiologies. a The 40b-to-20b ratio was used to segregate patients (mean ratio 0.78 ± 0.24) presenting with a significantly (p < 0.05) predominant 20 Hz anomaly (pink region; green squares; 20 Hz < 40 Hz; i.e. ON anomaly), 40 Hz anomaly (blue region; green triangles; 40 Hz < 20 Hz; i.e. OFF anomaly), or presenting with a 40b-to-20b ratio that is not significantly (p > 0.05) different (white region; green circles; $20 \approx 40$ Hz; i.e. normal-like) from normal (black circles; mean ratio 1.05 ± 0.06 ; N = 20). b ERGs of RP patients (red traces) and associated DWT scalograms representative of the three diagnostic classes (from left to right ON anomaly, normal-like and OFF anomaly). Representative ON-defect, ON-OFF defect and OFF defect-type RP ERGs are superposed to ERGs (black ERG traces) obtained (from left to right panel) either from a patient affected with congenital stationary night blindness (CSNB; i.e. ON anomaly), a normal subject or from a patient affected with congenital postreceptoral cone pathway anomaly (CPCPA; i.e. OFF anomaly). The red ERG tracings are associated with the left-hand side ordinates and the black ERG tracings with the righthand side ordinates.

Table 1: Normative data [sample size (N), mean and standard deviation (SD)] for every parameters measured on the patient along with the empirical and interpolated (between squared brackets) time point (in years) at which OS and OD permanently reached the statistical significance (p < 0.05) and the rates of changes of the different parameters measured in both eyes. The descriptors are sorted (from top to bottom) according to the

speed of detection measured in OD (the initially normal eye), so that the first descriptor that detected a significant pathological change (Hölder exponent) is at the top, and the last (Rod b-wave peak time) at the bottom.

12. Figures and Tables

Figure 1:



Figure 2:



Figure 3:







Figure 5:





Figure 7:



Figure 8:



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Figure 9:



Table 1:

Decade	Descriptor (unit)	Normative statistics				Empirical and [interpolated] time to permanently reach p < 0.05 (year)		Rate of change (unit/year)	
		N	Mean	SD	CV (%)	OS	OD	OS	OD
1st	DWT Hölder exponent (none) ^a	80	1.153	0.056	4.86	0 [0]	11 [6.9]	-0.017	-0.018
	TD Rod b-wave amplitude $(\mu V)^b$	25	202.11	33.21	16.43	0 [0]	11 [8.5]	-0.49	-3.92
	DWT 40 Hz b-wave (µV s) ^a	80	240.69	38.34	15.93	0 [0]	11 [9.9]	-2.18	-12.52§
2nd	DWT 20 + 40 Hz b-wave $(\mu V \ s)^a$	80	471.63	71.43	15.15	0 [0]	18 [13.1]	-6.11	$-25.04^{\$}$
	DWT 40 Hz a-wave (µV s) ^a	80	103.94	19.78	19.03	0 [0]	18 [13.5]	-1.78	$-4.68^{\$}$
	DWT 20 + 40 Hz a-wave $(\mu V s)^a$	80	182.12	32.2	17.68	0 [0]	18 [13.8]	-2.56	-9.2 [§]
	DWT 20 Hz b-wave (µV s) ^a	80	230.94	38.83	16.81	0 [0]	18 [15.0]	-3.92	-12.55
	DWT 20 Hz a-wave (µV s) ^a	80	78.18	14.92	19.08	0 [0]	18 [15.1]	-0.78	-4.52 [§]
	TD b-wave amplitude $(\mu V)^a$	80	98.93	21.3	21.53	0 [0]	18 [15.5]	-1.17	-5.68
	TD a-wave amplitude $(\mu V)^a$	80	30.14	7.05	23.39	0 [0]	18 [16.4]	-0.18	$-1.61^{\$}$
	Superior temporal vein (µm)	20	157.61	27.41	17.39	n/a	22 [19.1]	n/a	-4.7
	Inferior temporal artery (µm)	20	114.52	19.48	17.01	n/a	22 [19.2]	n/a	-3.1
	Superior temporal artery (µm)	20	105.21	20.34	19.33	n/a	22 [19.7]	n/a	-3.6
3rd	I4e visual field area (k pixel)	30	534.13	95.43	17.87	0 [0]	21 [20.2]	-2.95	-16.15§
	Inferior temporal vein (µm)	20	140.87	18.47	13.11	n/a	22 [20.5]	n/a	-2.9
	TD a-wave peak time (ms) ^a	80	13.16	1.13	8.59	22 [22.0]	22 [21.2]	0.18	0.3 [§]
	TD b-wave peak time (ms) ^a	80	31.19	1.18	3.78	22 [21.3]	22 [21.5]	0.22 [§]	0.14
	Visual acuity (none) ^c	n/a	0.8	n/a	n/a	0 [0]	24 [22.3]	-0.04	$-0.29^{\$}$
	TD Rod b-wave peak time (ms) ^b	25	70.32	4.23	6.02	Undetected	Undetected	0.01	0.01

[§] Rate of change that is significantly (p < 0.05) faster than the fellow eye</p>
^a Obtained from photopic ERGs at 0.64 log cd s m⁻²
^b Obtained from scotopic ERGs at -1.21 log cd s m⁻²

^c Considering the VA at onset as the reference

1. Preface to chapter V.

As above-mentioned, when the photopic ERG is evoked with short-duration stimuli (e.g. < 5 ms), the predominating activity of the ON-and OFF-pathways is blended to form the single b-wave we measure. As a result, the ON and OFF-pathways cannot be selectively measured with traditional standardized measurements of the standard short-flash ERG, therefore limiting its diagnostic power/utility. Of interest, in our second manuscript (Chapter III), the idea of a more selective representation of the retinal ON- and OFFpathways, trough the measurement of the 20 and 40 Hz components of the short flash photopic ERG, emerged according to the spectrum of DWT changes in diseases specifically affecting the ON- and OFF-pathways, respectively. Namely, the standard photopic ERG of patients with the complete type of congenital stationary night blindness (CSNB), a retinal disorder characterized by the ON-pathway dysfunction, presented with a marked attenuation of the 20 Hz component. On the contrary, in patients with congenital postreceptoral cone pathway anomaly (CPCPA), in which the retinal OFF-pathway is selectively affected, a severe reduction of the 40 Hz component was noted (Chapter III). The latter suggested that the 20 and 40 Hz components were more closely associated to the ON- and OFF-pathways, respectively. Similarly, in our third study (Chapter IV), more specific 20 and/or 40 Hz anomalies were reported in patients affected with Retinitis Pigmentosa, suggesting that the DWT could be instrumental in segregating various retinal dystrophy phenotypes or to more rapidly detect photopic ERG anomalies. However, the above findings were based on DWT analysis of the standard short flash ERG. In our fourth study (Chapter V), long duration photopic stimuli were analysed, because they allow a selective evaluation of the retinal ON- and OFF-pathways in the ON (b-wave) and OFF responses (d-wave), respectively. The purpose of our fourth manuscript is to assess the main frequency component of the ON (b-wave) and OFF response (d-wave) in the photopic long duration flash ERG and to observe the course of the ON and OFF responses across the spectrum of flash duration/strength. Our findings confirmed that the 20 and 40 Hz component of the photopic ERG mainly represents the ON- and OFF pathway, respectively.

2. Manuscript Title.

CHAPTER V

Manuscript 4 (Published Original Research Article)

QUANTIFYING THE ON AND OFF CONTRIBUTIONS TO THE FLASH ERG WITH THE DISCRETE WAVELET TRANSFORM

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3. Abstract

Purpose. Discrete wavelet transform (DWT) analyses suggest that the 20 and 40 Hz components of the short flash photopic ERG are closely related to the ON- and OFFpathways, respectively. With the DWT, we examined how the ERG ON and OFF components are modulated by the stimulus intensity and/or duration. Methods. DWT descriptors (20, 40 Hz and 40/20 Hz ratio) were extracted from ERGs evoked to 25 combinations of flash durations (150 to 5 ms) and strengths (0.8 to $2.8 \log cd.m^{-2}$). Results. In ERGs evoked to the 150 ms stimulus (to separate the ON and OFF ERGs), the 40/20 Hz ratio of ON ERGs (mean±SD: 0.49±0.04) was significantly smaller (p<0.05) than that of OFF ERGs (1.71 ± 0.18) , due to a significantly (p<0.05) higher contribution of the 20 and 40 Hz to the ON and OFF ERGs, respectively. With brighter stimuli, the ON and OFF components increased similarly (p<0.05). While progressively shorter flashes had no impact (p>0.05) on the ON component, it exponentially enhanced (p<0.05) the OFF component. Conclusions. DWT allows for an accurate determination of ON and OFF retinal pathways even in ERGs evoked to a short flash. To our knowledge, the significant OFF facilitatory effect evidenced with shorter stimuli has never been reported before. **Translational Relevance.** The DWT approach should offer a rapid, easy and reproducible approach to retrospectively and prospectively evaluate the function of the retinal ON and OFF-pathways using the standard (short flash duration) clinical ERG stimulus.

4. Introduction

The push-pull concept suggests that the genesis of the photopic ERG b-wave evoked to a flash of short-duration results from a balanced interaction between the retinal ON- and OFF-pathways.¹ Time-frequency analysis obtained using the discrete wavelet transform (DWT) suggested that the 20 Hz component of the photopic b-wave was more closely related to the activity of the ON-pathway, while the 40 Hz component appeared to mainly reflect the activity of the OFF-pathway. This claim was based on the fact that the 20 and 40 Hz components of the photopic b-wave were selectively attenuated in diseases differently affecting the ON and OFF pathways.²

When the photopic ERG is evoked in response to a flash of short-duration (e.g. <5 ms), activities of the ON and OFF pathways are merged to form the response we record. However, with stimuli of longer duration (e.g. >150 ms), a distinct OFF component, known as the d-wave, is seen at the offset of the stimulus.^{1, 3, 4} Studies in non-human primates revealed that the b-wave of the long-flash ERG mostly originated from ON-bipolar cells activity¹ while the source of the d-wave was mostly attributed to OFF-bipolar cell activity.⁴

Based on the above, the purpose of this study is to determine the time-frequency composition of the ERG ON and OFF responses separately and how each response is modulated by the intensity and/or the duration of the stimulus.

5. Methods

This study was performed on 10 normal subjects (one male and nine females aged 23–43 years old; mean age 31.9 years). The research was conducted according to the tenets of the Declaration of Helsinki and was approved by the National Ethics Committee. All subjects signed an informed consent form previously approved by the Institutional Review Board. The IRB also allowed us to make use of archived clinical ERG data for which the acquisition of a clear informed consent could not be readily demonstrated. In all cases, subjects provided consent to the ERG procedure.

5.1 ERG recordings

Electroretinogram recordings were performed with an Espion visual electrophysiology testing system (Diagnosys LLC, Littleton, MA). The recording electrode (HK-loop⁵) was hooked to the lower lid as previously reported elsewhere.^{6,7} The reference and ground electrodes (Grass silver cup electrodes; Grass Technologies, West Warwick, RI) were pasted at the level of the external canthus and on the forehead, respectively. The pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon, Inc., Fort Worth, TX). Following a 10-minute period of light adaptation, photopic ERGs were elicited with a Ganzfeld ColorDome stimulator (Diagnosys LLC). Broadband white stimuli were delivered against a white 20-cd.m⁻² rod desensitizing background as previously documented.^{6,7} Flash strength was gradually increased in steps of 0.5 log units, covering a range of 6.3 to 630 cd.m⁻² (i.e., 0.8–2.8 log cd.m⁻²). These luminance series were recorded using flash durations of 5, 10, 20, 50, and 150 ms. Flashes were delivered at 1-Hz intervals, and 20 responses were averaged to yield a single waveform. The signals were recorded with a bandpass filter (bandwidth: 0.15–300 Hz), exported as ASCII files, and imported in Matlab R2015a (Mathworks, Natick, MA) for further analysis.

5.2 Wavelet analysis

According to a previously published method,^{2,8,9} the DWT of each ERG signal was computed using the fast wavelet transform algorithm¹⁰ implemented with Matlab R2015a and adapted to achieve shift invariance. In order to reduce edge effects, before DWT computation, each response was padded with 512 additional samples (by repeating the first and last value 256 times on both sides of the signal). Discrete wavelet transform scalograms were then normalized to the maximal coefficient. Each scalogram included eight frequency bands, each with a distinct central frequency (10, 20, 40, 80, 160, 320, 640, and 1280 Hz; see Fig. 1A), where each band quantified the contribution of a range of components oscillating around the central frequency. This time-frequency approach allows for the identification of energy descriptors, each defined by their respective time and frequency coordinates. As previously suggested, the DWT could potentially yield measurements of
the ON and OFF components of the ERG (local wavelet maxima found in the 20- and 40-Hz bands, respectively) through the quantification of their respective associated wavelet coefficients.² In the present study, this claim is further investigated by assessing the major time-frequency components of the ON and OFF ERGs evoked to long-flash (i.e., 150 ms) stimulus. Finally, numerous mother wavelets can be used to extract the time-frequency components of the ERG (e.g., 20-Hz and 40-Hz components). In this study, we wished to analyze ERG components that were well-localized in time (such as the b- and d-waves). The Haar wavelet (which has a square wave-like shape) was chosen not only for its simplicity but also because, among all the wavelets considered, it is the one that provided the highest temporal accuracy; the Haar wavelet thus has the ability to optimally identify the local components of the ERG signal.² Furthermore, the Haar wavelet has the shortest wavelet filter length, making it less prone to contamination from neighboring components (such as a-wave contamination on the b-wave). Additional rationales for the use of this mother wavelet were also discussed elsewhere.²

5.3 Statistical Analysis

Two-way analysis of variance (ANOVA) was used to evaluate the interaction of the two main effects (i.e., stimulus duration and strength) on the ON and OFF ERG components. When the interaction effect was significant, a post hoc analysis was conducted using Bonferroni correction for multiple comparisons. Finally, Pearson correlations were used to assess relationships between selected variables.

6. Results

Representative ERG tracings evoked to each of the 25 combinations (i.e., 5×5) of stimulus strength and duration used in this study are shown in Figure 1 as an average of all the waveforms obtained from all 10 subjects. One can note that with the shortest (5 ms) flash duration (top tracings), the amplitude of the b-wave increases steadily with progressively stronger stimuli (from left to right) to reach the largest amplitude with a flash luminance of 2.8 log cd.m⁻². When increasing the flash duration to 10 and 20 ms, the peak amplitude of the b-wave was reached with a 0.5 log-unit dimmer stimulus (2.3 log cd.m⁻²).

A d-wave (OFF response) could only be seen (red arrows) in ERGs evoked to a 50-ms or greater stimulus (after the offset of the stimulus).

6.1 DWT Components of ON and OFF Responses

The longest stimulus duration (150 ms) combined to the brightest flash luminance (2.8 log cd.m⁻²) optimally separated the b- and d-waves (i.e., ON and OFF ERGs, respectively) in all subjects. Responses obtained using this stimulus condition were used to assess the main frequency component of the ON and OFF ERGs, respectively. As shown with the DWT scalograms reported in Figure 2A, ON ERGs are characterized with a predominant 20-Hz component (white arrows), with the same timing as the b-wave, whereas OFF ERGs are characterized with a strong 40-Hz component (white arrows) that is time-locked to the d-wave. This time-frequency signature remains quantifiable in ERGs evoked to progressively dimmer stimuli and even in noisy (blink and/or muscular twitch corruption) recordings (see the bottom tracings of Fig. 2B). In this study, the ON response was therefore quantified by identifying the maximal 20-Hz component that immediately followed the onset of the stimulus, while the OFF response was quantified by measuring the maximal 40-Hz component that immediately followed the offset of the stimulus.

As shown in Figure 3A–B, the ON ERG waveform evoked at the onset of the 150ms stimulus was algebraically subtracted from ERG waveforms evoked to stimuli of shorter durations (i.e., 5, 10, and 20 ms), where both the ON and OFF ERG components are mixed. This was done to extract the hidden OFF ERG components (shown in Fig. 3B). The corresponding DWT scalograms (reported at the right of the OFF ERGs in Fig. 3B) confirm that the extracted OFF ERGs are also dominated by a 40-Hz component (white arrows). Of note, the 40-Hz component isolated algebraically is slightly (but not significantly; P > 0.05) less energetic (mean of all extracted OFF ERG energy: 127.36 μ V.s) compared to that included in the short-flash (ON-OFF) ERG responses (mean of all: 149.93 μ V.s). However, as shown in Figures 3C–E, this algebraically isolated OFF ERG component was highly (Pearson scores of up to 0.97) and significantly (P < 0.05) correlated with the 40-Hz component directly measured in its scalogram accurately quantify the OFF ERG contribution to the making of the short-flash ON-OFF ERG waveform.

6.2 Stimulus Strength/Duration-Dependence on the 20 and 40 Hz Components

The stimulus strength/duration-dependence (mean \pm SD) of the 20-Hz and 40-Hz ERG components are displayed in Figure 4A and B, respectively. As shown in Figure 4A, with progressively brighter stimuli (irrespective of stimulus duration), the 20-Hz component (ON response) regularly increases to reach a maximal value with a flash of 2.8 $\log \text{ cd.m}^{-2}$ in strength. In contrast, a gradual shortening of the flash duration from 150 to 5 ms, minimally impacts the energy level of the 20-Hz component (averaged variation [i.e., range divided by mean] of 37.22%). The maximal values were reached with the 10-ms flash, irrespective of stimulus strength. The relatively small effect of the stimulus duration on the 20-Hz component is confirmed with the two-way ANOVA statistic, where the F value for the duration effect (F = 6.6; P < 0.05) was 75 times smaller than that of the stimulus strength effect (F = 494.1; P < 0.05). Although both main effects significantly (P < 0.05) modulated the 20-Hz component, the interaction effect was not significant (F = 0.79; P = 0.69), and consequently, no further statistical analyses (i.e., post hoc analyses) were conducted. A different picture emerged with the 40-Hz component (OFF response). As shown in Figure 4B, progressively brighter stimuli increased the 40-Hz component to a maximal value reached with a flash of 2.3 log $cd.m^{-2}$ in strength, except for the shortest stimulus duration where the maximal value was reached with the 2.8 log cd.m⁻² stimulus. Similarly, a progressive shortening of the flash duration from 150 to 5 ms always enhanced this component, albeit to different extents. For example, for the brightest stimulus (red curve), shortening the flash duration from 150 to 5 ms enhanced the 40-Hz component from 20.41 to 198.63 µV.s, representing an increase of 973.21% compared to one of 122.71% when the dimmest stimulus is used. The latter indicates that the enhancement of the OFF component seen with the shorter stimuli is intensity dependent. This claim is best illustrated in Figure 5A, where this facilitatory effect of the OFF component witnessed with progressively shorter stimuli is plotted against the stimulus strength. As shown, this facilitatory effect (i.e., a value greater than 100%) was seen irrespective of the intensity of the stimulus strength. However, the strength of the stimulus exponentially amplified this

effect. Consequently, in contrast to the 20-Hz component, both main effects (stimulus duration and strength) of the 40-Hz OFF component were strong. This is best exemplified with the two-way ANOVA statistic with F values of 153.5 and 400.7 (P < 0.05) for stimulus duration and strength, respectively. The interaction effect was also found to be significant (F = 31; P < 0.05). Post hoc analyses revealed that flashes of 50, 20, 10, and 5 ms significantly (P < 0.05) increased (as marked by the colored asterisks) the 40-Hz energy (compared to values obtained with flashes of 150 ms) for stimulus strength of 1.3, 1.8, 2.3, and 2.8 log cd.m⁻², but not for the dimmest stimulus (0.8 log cd.m⁻²).

Finally, as shown in Figure 5B, summating the 20- and 40-Hz components of the short-flash (SF: 5 ms) ERG or of the 20- and 40-Hz component of the ON and OFF components of the long-flash (LF: 150 ms) ERGs resulted in similar values (SF: 19.57 μ V.s; LF: 22.06 μ V.s; P > 0.05) in ERGs evoked to the dimmest flash (0.8 log cd.m⁻²), but resulted in progressively larger differences when using brighter stimuli (e.g., SF: 378.78 μ V.s; LF: 193.04 μ V.s; P < 0.05 with the 2.8 log cd.m⁻² stimulus). These results would suggest that short-flash ERGs evoked to the dimmest stimulus would result from a simple summation of the ON and OFF components, while with brighter stimulus strengths, this simple addition would not be enough to explain the resulting ERG response, most probably due to the OFF facilitatory effect evidenced at Figure 5A.

6.3 Correlations Between Frequency Components of Short and Long-flash Responses

The relationship between 20-Hz and 40-Hz components of the ERG responses evoked by long and short stimuli in the 10 subjects is presented in Figure 6. As exemplified in Figure 6A, there is a strong correlation (r = 0.76; P < 0.05) between the energy level of the 20-Hz component of the short-flash ERG and the 20-Hz component of the ON ERG evoked to the long-duration stimulus. An even stronger correlation (r = 0.90; P < 0.05) was found between the 40-Hz component of ERGs evoked to the short stimuli and the 40-Hz of the OFF ERGs evoked by long-duration stimuli (Fig. 6B). However, no correlation could be found between the 20-Hz component of short-flash ERGs and the 40-Hz component of long-flash OFF ERGs (Fig. 6C) or between the 40-Hz component of short-flash ERGs and the 20-Hz component of long-flash OFF ERGs (Fig. 6D).

7. Discussion

Our results show that the DWT of photopic ERGs evoked to stimuli of long duration allows for the identification of distinct frequency components where the ON ERG is mostly dominated by a 20-Hz component and the OFF ERG by a 40-Hz component, thus confirming previously published findings.² Consequently, as presented in Figure 7A, the 40:20-Hz energy ratio was smaller than 1 for the ON response (0.49 ± 0.04) and greater than 1 for the OFF response (1.71 ± 0.18) . These values are reminiscent of the 40:20-Hz energy ratio previously reported² (see also Fig. 7B) for the ERGs of patients affected with an OFF-specific anomaly (0.43 ± 0.06) ; congenital postreceptoral cone pathway anomaly [CPCPA] patients^{11,12}) or an ON-specific anomaly $(2.01 \pm 0.30; \text{ congenital stationary night})$ blindness [CSNB] type 1 patients^{13–15}). Taken together, our findings support the claim that the 20- and 40-Hz components of the short-flash photopic ERG are intimately linked to electrical events within the retinal ON and OFF pathways, respectively. Furthermore, we showed a significant (P < 0.05) correlation (Fig. 6) between the 20-Hz component of the short- and long-flash ERGs as well as between the 40-Hz component of the short- and longflash ERGs, raising the possibility that, irrespective of flash duration, both components would be generated by the same retinal pathway (such as ON and OFF bipolar cells, 20 and 40 Hz, respectively), a claim that needs to be further investigated.

ON and OFF responses of the photopic ERG can be easily delineated with the use of long-duration stimuli, while these two components blend in ERGs evoked to shorter (< 50 ms) stimuli.^{16–19} Of interest, the DWT permitted us to quantify the ON and OFF responses based on their separable time-frequency features and therefore unmasked ON-OFF interactions that would have otherwise remained unquantifiable (Fig. 4). While the 20-Hz component of the ON response increases gradually with brighter stimuli, the duration of the stimulus appears to negligibly impact this component (Fig. 4A). In contrast, both flash strength and duration seem to positively impact the 40-Hz component of the OFF ERG (Fig. 4B).

Other authors previously studied ON-OFF interactions within the short-flash ERG. Earlier studies assumed that there was a simple algebraic addition of the two components,²⁰ but this concept was later shown to hold only for ERGs evoked to dim flashes, as selected

brighter flashes enhanced the OFF response.21 Similar to what was previously proposed by Howarth (1961), our findings also suggest a simple summation of the ON and OFF components for ERGs evoked to low stimulus strengths (see Fig. 5B).²⁰ However, confirming the claim of Walters et al.,²¹ our findings also suggest that for the short-duration ERGs evoked to brighter flashes, the algebraic addition no longer works (see Fig. 5B), most probably due to the exponential enhancement of the OFF response (see Fig. 5A). Supporting the latter are findings in patients with an ON-specific anomaly (CSNB) which clearly suggested a facilitating contribution of the OFF pathway in ERGs evoked to bright, short-duration, flashes.^{7,22} Similarly, Kondo et al.¹⁷ showed that reconstructed ERGs obtained by adding the ON and OFF (b-wave + d-waves of a 250-ms ERG response) ERG responses, yielded a waveform that was consistently smaller in amplitude than that measured for the short-flash (5 ms) ERG, where the ON and OFF components are fused together.¹⁷ We believe that the above findings find an explanation in our demonstration of the OFF facilitatory effect. Finally, similar to what was previously advanced, ^{2,11,17,23–25} our study further confirms that the OFF pathway significantly contributes to the short-flash ERG. The OFF facilitatory effect could add diagnostically meaningful information to the clinical ERG, a claim that will, however, require further testing. Finally, it is worth remembering that our claims are based on a flash stimulus in which the light is turned ON first and then turned OFF. It would be interesting to investigate if similar conclusions would be reached when the short-flash ERG response is evoked to an OFF-ON stimulus, where the OFF component precedes the ON component.

In conclusion, our results clearly demonstrate that use of the DWT allows for an easy, fast, and reproducible quantification of the ON and OFF retinal pathways using a short-flash ERG, which is the most widely used stimulus in clinical electrophysiology, as it is the standard ERG stimulus prescribed by the International Society for Clinical Electrophysiology of Vision (ISCEV).²⁶ The addition of this novel analytical tool to the armamentarium of retinal electrophysiologists should therefore prove useful in diagnosing, classifying, and staging retinal disorders impairing the retinal function postreceptorally, as an imbalance between the ON and OFF retinal pathways were previously shown to characterize some retinal dystrophies (see Sieving³ for review).

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10. Captions of figures

Fig. 1. Representative ERG traces. Composite ERG signals that were obtained (by averaging the responses of all 10 subjects) using short (5 ms) and long (10, 20, 50 and 150 ms) white flash stimuli of various strengths (0.8, 1.3, 1.8, 2.3, 2.8 log cd.m⁻²) against a white background of 20 cd.m⁻². Vertical black arrows indicate the onset of the stimulus, while black horizontal lines indicate the luminous phase of the stimulus. Red arrows indicate the OFF responses (d-waves). The vertical calibration bar (75 μ V) applies to each trace. Traces evoked to the 0.8 log cd.m⁻² stimulus were magnified by a factor of 2 for visualization purposes.

Fig. 2. Main time-frequency component of ON and OFF responses. (A) Three examples of representative ON and OFF response traces, along with their associated DWT scalograms, obtained using a long (150 ms) white flash stimulus of 2.8 log cd.m⁻² in strength. Each ON response is characterized by a strong 20-Hz component (white arrows) that is time-locked to the b-wave. Each OFF response is characterized by a strong 40-Hz component (white arrows) that is time-locked to the d-wave. Each scalogram is normalized to the maximal coefficient (0%–100%). (B) Representative ON and OFF responses, elicited with progressively dimmer flashes (from 2.3 to 0.8 cd.m⁻²), demonstrating that 20- and 40-Hz components dominated ON and OFF responses, respectively, independently of the stimulus strength. This was not evident for the dimmest flash, where the response was almost at the level of noise.

Fig. 3. Main time-frequency component of algebraically extracted OFF responses. (A) Electroretinogram signals obtained by averaging the responses of all 10 subjects and acquired using short-duration stimuli of 5, 10, and 20 ms (identified as F5, F10, F20) where

both the ON and OFF responses are merged (ERG = ON + OFF) and a long-duration stimulus of 150 ms (identified as F150) with separated ON and OFF responses. Associated DWT scalograms are shown on the right-hand side of the ERG tracings and are normalized to their maximal coefficients (0%–100%). (B) Algebraically extracted OFF responses obtained by subtracting the long-flash (F150) ON ERG from the mixed ON-OFF ERGs (F5, F10, F20). Associated DWT scalograms are shown on the *right-hand side* of each tracing. The dominant 40-Hz component contained within these OFF responses is indicated by white arrows. (C–E) Correlations between the 40-Hz component of the mixed ON-OFF ERGs (ordinate) and the 40-Hz component of the algebraically extracted OFF responses (abscissa). Pearson coefficients (r) and associated P values are given in each panel.

Fig. 4. Duration/luminance-dependence of 20 and 40 Hz components. (A) Mean (\pm SD) 20-Hz energy obtained using progressively brighter stimulus strength (0.8: blue curve; 1.3: green curve; 1.8: yellow curve; 2.3: orange curve; 2.8: red curve) and progressively shorter stimulus (from 150 to 5 ms). (B) Mean (\pm SD) 40-Hz energy obtained using progressively brighter stimulus strength same color coding as Panel A) and progressively shorter stimulus (from 150 to 5 ms).

Fig. 5. Stimulus strength dependence of the OFF facilitatory effect. (A) Enhancement of the OFF facilitatory effect (i.e., increase in the energy level of the ERG OFF component with progressively shorter stimuli) with progressively brighter stimuli (abscissa). The OFF facilitatory effect was obtained by dividing the 40-Hz energy level measured in the short-flash (i.e., 5 ms) ERG with that measured in the long-flash (i.e., 150 ms) ERGs multiplied by 100. (B) Comparison between the summation of the 20- and 40-Hz components of the short-flash (SF: 5 ms) ERG (blue bars) with the summated 20- and 40-Hz components of the ON and OFF ERGs evoked to a long-flash (LF: 150 ms) stimuli (red bars) of increasing intensities (as indicated at the bottom of each bar graph).

Fig. 6. Correlations between short and long-duration flash responses. (A) Correlation between the 20-Hz component of the SF ERG and the 20-Hz component of the ON response of the long-flash ERG. (B) Correlation between the 40-Hz component of the short-flash ERG and the 40-Hz component of the OFF response of the long-flash ERG. (C) Correlation between the 20-Hz component of the short-flash ERG and the 40-Hz component of the short-flash ERG and the 40-Hz component of the short-flash ERG.

component of the OFF response of the long-flash ERG. (D) Correlation between the 40-Hz component of the short-flash ERG and the 20-Hz component of the ON response of the long-flash ERG. Coefficients of correlation (r) and associated P values are indicated on each graph. Nonsignificant P values are indicated as NS. In panels A to D, the *black circles* represent the data points from each of the 10 subjects.

Fig. 7. Comparison of normal ON and OFF responses with CPCPA and CSNB responses. (A) The 40:20-Hz energy ratio was used to segregate the long-flash (LF) ON responses (LFON; *blue circles*) from the LF OFF responses (LFOFF; *red circles*). Representative LFON and LFOFF responses and the corresponding DWT scalograms are shown at the bottom of panel A. (B) A similar 40:20-Hz energy ratio was obtained from the short-flash ERGs recorded in patients presenting with an OFF-specific anomaly (CPCPA; *blue circles*) or an ON-specific anomaly (CSNB; *red circles*). Representative CPCPA and CSNB ERGs and corresponding DWT scalograms are shown at the bottom of panel B. In the scalograms, the highly energetic *dark-red coefficients* (indicated as 20 Hz or 40 Hz) further demonstrate the dominant 20- or 40-Hz component measured in LFON and CPCPA ERGs or in LFOFF and CSNB ERGs, respectively.

11. Figures

Figure 1



Stimulus strength (log cd.m⁻²)





Figure 3







Figure 5



Figure 6



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Figure 7



1. Preface to chapter VI.

In the above four Chapters (Chapters II to V), it was shown that advanced methods can be used to extract valuable information to complement the traditional, centuries-old, analyses of the ERG. Among advanced approaches, the discrete wavelet transform stood out as the best and easiest way to derive relevant descriptors from the time-frequency domain representation of the ERG (Chapter II). Several DWT descriptors, quantifying local energy and/or signal complexity, were defined (Chapters II and III) and their basic and clinical usefulness proofed and put to the test (Chapters III to V). While descriptors concomitant with the fast components of the ERG (i.e. the oscillatory potentials: OPs) were identified (e.g. ops80, ops160), our clinical applications and demonstrations mainly focused on the slow-frequency components of the ERG (e.g. 20a, 40a, 20b, 40b, etc.). At this point, several questions remain to be elucidated regarding the OPs. For example, is the contribution of OPs to the building of the ERG invariable and remain stable irrespective of the stimulus intensity and/or amplitude of the signal? In other words, does the ERG energy and OPs energy goes hand in hand? Similarly, in pathological conditions, the highfrequency components can be preferably affected, but is it also true of the slow-frequency components? Can an ERG present with predominating high-frequency components? Traditionally, the OPs are measured following bandpass filtering, but the latter approach can introduce phase lags and uneven attenuation/enhancement of OPs. Can the use of the DWT be generalized to derive more reproducible information from the ERG and answer some of the above questions? In order to address these research questions, our last manuscript (Chapter VI) was strictly limited to the analysis of the OPs, and their contribution to the building of the ERG. We found a relatively constant and highly reproducible contribution of OPs in normal subjects, and a significantly wider range of %OP values in patients, with some presenting with ERGs that are almost solely composed of OPs. The DWT therefore represents a useful approach to help in the segregation of pathological ERGs based on the relative weight of OPs in the ERG.

2. Manuscript Title.

CHAPTER VI

Manuscript 5 (Published Original Research Article)

ASSESSING THE CONTRIBUTION OF THE OSCILLATORY POTENTIALS TO THE GENESIS OF THE PHOTOPIC ERG WITH THE DISCRETE WAVELET TRANSFORM

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3. Abstract

The electroretinogram (ERG) is composed of slow (i.e., a-, b-waves) and fast (i.e., oscillatory potentials: OPs) components. OPs have been shown to be preferably affected in some diseases (such as diabetic retinopathy), while the a- and b-waves remain relatively intact. The purpose of this study was to determine the contribution of OPs to the building of the ERG and to examine whether a signal mostly composed of OPs could also exist. DWT analyses were performed on photopic ERGs (flash intensities: -2.23 to 2.64 log cd·s·m⁻² in 21 steps) obtained from normal subjects (n=40) and patients (n=21) affected with a retinopathy. In controls, the %OP value (i.e., OPs energy/ERG energy) is stimulus-and amplitude-independent (range: 56.6–61.6%; CV = 6.3%). In contrast, the %OPs measured from the ERGs of our patients varied significantly more (range: 35.4%–89.2%; p < 0.05) depending on the pathology, some presenting with ERGs that are almost solely composed of OPs. In conclusion, patients may present with a wide range of %OP values. Findings herein also support the hypothesis that, in certain conditions, the photopic ERG can be mostly composed of high-frequency components.

4. Introduction

The electroretinogram (ERG) waveform is characterized with a negative a-wave that is generated by the photoreceptors followed by a larger positive b-wave, which originates from the bipolar and Müller cells [1]. Low-voltage high-frequency oscillations, known as the oscillatory potentials (OPs), are also often seen riding on the ascending limb of the b-wave [2, 3]. Although their origin remains debated, it has been suggested that they would represent inner retinal potentials generated by neuronal interactions that might involve bipolar, amacrine, and/or ganglion cells [3–6]. Of interest, while previous studies have shown that, in some retinopathies (such as in diabetic retinopathy or central retinal vein occlusion), the OPs appeared to be selectively more affected compared to the relatively better preserved a- and b-waves [7–9], to date no study has reported ERG responses which seemed to be solely composed of OPs or where the OPs appeared to be selectively better preserved than the slower components of the ERG (i.e., a- and b-waves).

Notwithstanding the above, it must be remembered that traditionally the OPs are extracted using a bandpass filtering technique in order to remove the low-frequency components (i.e., a- and b-waves) from the broadband ERG signal. Unfortunately, bandpass filtering can generate signal distortion such as phase lag, ringing artefacts, and/or attenuation of OP amplitude which can lead to erroneous measures and even, in some instances, "create" artifactual OPs [10, 11]. As a remedy to the latter, it was suggested to quantify the OPs in the frequency domain with the use of the fast Fourier transform (FFT) [6, 12]. Unfortunately, given that the FFT quantifies the power level of all the frequency components contained within a signal (such as the ERG), whether they are time-locked to the stimulus or not (i.e., no temporal resolution), its use can lead to erroneous interpretations [10, 13–15]. Fortunately, the latter limitation can be easily overcome with the use of the discrete wavelet transform (DWT), which is somewhat of an improved FFT since it includes both temporal and frequency resolutions [10, 13–18].

With the above in mind, we sought to determine the contribution of OPs to the building of the photopic ERG waveform obtained from normal subjects and patients and to examine whether a signal mostly composed of OPs could also exist.

5. Materials and Methods

5.1 Selection of ERG Responses Analysed

Analysis was performed on photopic ERGs (bandwidth: 1-300 Hz; flash intensities: -2.23 to 2.64 log cd·sec·m⁻² in 21 steps of ~0.2 log-unit; background: $30 \text{ cd} \cdot \text{m}^{-2}$; averages of 10 to 300 flashes per response) obtained from 40 normal subjects. Results were compared to photopic ERG responses obtained from patients (bandwidth: 1-300 Hz; flash intensities: 0.64 log cd·sec·m⁻²; background: $30 \text{ cd} \cdot \text{m}^{-2}$; averages of 10 to 300 flashes per response) affected with retinopathies known to selectively abolish the OPs [i.e., diabetic retinopathy (DR) and central retinal vein occlusion (CRVO)] [7–9]. These patients were selected on the basis of the clinical findings that were characteristic of the disease condition (i.e., mostly fundus appearance and in the case of CRVO, unilateral presentation). Results were also compared to photopic ERG response obtained from patients (bandwidth: 1-300 Hz; flash intensities: 0.64 log cd·sec·m⁻²; background: 30 cd·m⁻²; averages of 10 to 300 flashes per response) affected with an advanced retinal degeneration (mostly retinitis pigmentosa) and where, on visual inspection (by a naïve observer: CS), fast oscillations, time-locked to the stimulus and in the frequency range of the OPs (as estimated using a template of a normal OP response) appeared to be the most prominent features of the response. An informed consent form was signed by each subject and the protocol was previously approved by the Institutional Review Board and was conducted in accordance with the declaration of Helsinki. Additional details regarding the ERG setup and recording procedures were previously published by us [19–23].

5.2 Analysis of ERG Responses

The DWT of each ERG signal was computed using the fast wavelet transform algorithm of Mallat [35] implemented with Matlab R2015a. The DWT generates scalograms (Figure 1(a)) which display the energy (z-axis) of the signal (maximal values are shown in red; lowest values in blue) as a function of time (x-axis) and frequency (y-axis). As previously demonstrated by us and others [10, 13, 15–18], this time-frequency approach allows for the identification of energy descriptors, each defined with their respective time and frequency coordinates. The DWT yields measurements of the photopic

b-wave (found in the 20 and 40 Hz bands) and OPs (found in the 80 and 160 Hz bands) through the quantification of their respective associated wavelet coefficients [10, 16, 17]. As exemplified in the scalogram of Figure 1(a), two DWT descriptors were used to quantify the 20 Hz and 40 Hz b-wave energy (identified as 20b and 40b) and another two were used to quantify the 80 Hz and 160 Hz OPs energy (identified as 80ops and 160ops), each computed by summating values outlined by the white boxes, respectively. Similar to bandpass filtering, the inverse DWT (IDWT; see [10, 17]) can be used to specifically reconstruct the low-frequency (20 and 40 Hz) and high-frequency (80 and 160 Hz) bands of the signals, which are specific to the slow and fast waves of the ERG. As shown in Figures 1(c) and 1(d), the IDWT confirms that the 20 and 40 Hz descriptors quantify the slow waves (a- and b-waves, as identified in Figure 1(c)) of the ERG, while the 80 Hz and 160 Hz descriptors quantify the fast waves (OPs, identified as 2, 3, and 4 in Figure 1(d)). Consequently, these DWT descriptors were used to quantify the percent contribution of the OPs (%OPs) to the ERG according to the following equation:

$$\% OPs = \frac{OPs \, Energy}{Broadband \, ERG \, Energy} = \frac{80 ops + 160 ops}{20b + 40b + 80 ops + 160 ops} \tag{1}$$

5.3 Statistical Analysis

Descriptive statistics (mean and standard deviation [SD]) of the %OPs were obtained from our selected patients and from our normal subject cohort at each of the 21 stimulus intensities. The coefficient of variation (CV) of selected groups was computed as the SD divided by the mean and multiplied by 100. To further confirm that each of our selected pathological ERGs had a significantly (p < 0.05) lower (DR and CRVO patients) or higher (end-stage retinal degeneration patients) than normal %OPs, individual one-way -tests were used with a critical value set at 1.645 [36]. Thus Z-scores of \pm 1.645 will indicate a significantly higher/lower (p < 0.05) than normal contribution of the OPs to the broadband ERG, respectively. Finally, an unpaired t-test (one-tail) was also used to validate whether our group of advanced retinal degeneration patients had a significantly (p < 0.05) higher percentage of OPs (%OPs) contributing to their ERGs.

6. Results

6.1 %OPs Contribution to the Normal ERG Response

Representative ERG waveforms of the photopic luminance-response (LR) function obtained at four different stimulus intensities are shown in Figures 2(a) to 2(d) and the positions of these responses on the photopic LR function are indicated with a red diamond in Figure 2(e). As shown in Figure 2(a), no OPs could be detected on the rising phase of the b-wave evoked to the dimmest stimulus (i.e., $-1.41 \log \text{ cd} \cdot \text{m}^{-2}$) as well as in the response (Figure 2(b)) evoked to a stimulus one log-unit brighter ($-0.41 \log \text{ cd} \cdot \text{m}^{-2}$), despite a 10-fold increase in b-wave amplitude (i.e., from ~ 3 to $\sim 30 \,\mu\text{V}$). The latter contrasts with the ERG evoked to the 0.64 log $cd \cdot s \cdot m^{-2}$ stimulus (Figure 2(c)) where two prominent OPs are seen on the ascending phase of the b-wave. A further increase in stimulus intensity to 2.39 log $cd \cdot s \cdot m^{-2}$ (Figure 2(d)) will yield a b-wave of reduced amplitude (approximately 30 uV) compared to the 0.64 log cd·s·m⁻² response but with more OPs. The above suggests, at least from visual inspection, that the prominence of OPs increases with stimulus intensity, but not necessarily with b-wave amplitude. However, as indicated at the top of the four representative scalograms (Figures 2(a)-2(d)), when we measure the %OPs content of these ERGs, nearly identical values are found (%OPs = 58.7; 57.8; 56.5; and 59.4 for intensities of -1.41; -0.41; 0.64; and 2.39 log cd s m⁻², resp.). This is best exemplified with the graphs of Figures 2(e) and 2(f) reporting the luminanceresponse functions of DWT descriptors of the b-wave (i.e., 20b + 40b; gray trace; diamond markers) and OPs (i.e., 80ops + 160ops; black traced; round markers) are nearly identical (once normalized). Therefore, as reported in Table 1 and illustrated in Figure 2(f), in normal subjects, across all of the 21 stimulus intensities (close to 5 log-unit range), the %OPs varied between 56.6 (smallest value) and 61.6 (highest value) and was thus significantly less variable than the b-wave amplitude which varied between 1.2 μ V (lowest value) and 115.52 μ V (highest value). The above demonstrate not only that the %OPs is nearly stimulus-independent but also that it varies very little (see Table 1) as the mean %OPs coefficient of variation (CV) of all the intensities considered was of 6.34% compared to 87.37% for the b-wave amplitude.

6.2 Pathological ERG Responses Presenting with a Reduced %OPs Contribution

As predicted from previous studies [7–9], ERGs recorded from patients affected with diabetic retinopathy (DR, Figure 3(b)) or central retinal vein occlusion (CRVO, Figure 3(c)) presented no evidence of OPs on the rising phase of the b-wave. These conditions allowed us to investigate if our method of determining the OP content with the DWT (i.e., the %OPs) would be reduced in situations where the OPs are selectively attenuated. The latter is clearly reflected with the significantly reduced %OPs of 35.4% (Z-score: -8.48; p <0.05) and 36.7% (Z-score: -7.96; p < 0.05) that we found for DR and CRVO, respectively.

6.3 Pathological ERG Responses Presenting with a Higher %OPs Contribution

Analysis of ERGs obtained from our databank revealed that the patients in this group were mostly affected with retinitis pigmentosa (RP), accounting for a total of 15 cases. The clinical findings such as, visual fields, visual acuities, rod ERG amplitudes, which are reported in Table 2, are indicative of end-stage retinopathies. Using the abovedefined DWT technique, we determined that the overall percentage of OPs contribution was significantly greater than that obtained from control (%OPs of $74.1\% \pm 8.1\%$ versus $56.6\% \pm 2.5\%$, resp., p < 0.05) using the same suprathreshold photopic ERG waveform (0.64 log cd.s.m⁻²). Representative examples of enhanced %OPs ERGs are shown in Figure 4 and data from patients are reported in Table 2. For example, as shown in Figure 4(a), Patient 1 (also referred as Patient 1 in Table 2) presented with a %OPs of 61.1%, a value that is slightly but nonetheless significantly higher than control (Z-score: 1.78; p < 0.05). In contrast, the ERGs of Patients 2 and 3 (Figures 4(b) and 4(c)), presented with a more pronounced enhancement of the %OPs parameter to 75.4% and 78.4%, respectively (Zscores: 7.46 and 8.67; p < 0.0001). Finally, the ERG tracing and scalogram obtained from Patient 4 (Figure 4(d)) disclosed a waveform that is almost solely composed of OPs with a %OPs of 89.2% (Z-score: 13.14; p < 0.0001), the highest %OPs value obtained from our patient cohort.

6.4 Example of %OPs Progression with Disease Process

Figure 5(a) shows the photopic ERGs obtained from two brothers (aged 12 and 17 years at initial exam and identified as patients 16 and 17, resp., in Table 2) affected with

choroideremia. On initial examination, both brothers presented with severely attenuated ERGs, albeit with normal looking morphologies, that contrast with the more oscillatory ERG waveforms that we obtained seven years later. The corresponding DWT scalograms indicate that while, at the initial visit, the %OPs was within the normal limits (i.e., 56.2% and 55.1%), seven years later it had increased to 65.2% and 79.9%, suggesting that disease progression was most detrimental to the slow frequency generators of the ERG (e.g., b-wave).

7. Discussion

The purpose of this study was to determine the energy contribution of the oscillatory potentials to the building of the photopic ERG response and to investigate if an ERG could be mostly composed of OPs. Our study demonstrated that, in normal ERGs, the %OP value is relatively constant (overall CV = 6.3%), not stimulus-dependent (range 56.6% to 61.6%), and consequently not influenced by the absolute amplitude of the ERG b-wave (which varied between 1.2 and 115.4 μ V; overall CV = 87.4%). This nearly stimulus-independent %OPs value can be explained by the fact that the b-wave and OPs covary across the luminance-response function of the normal photopic hill, increasing in the initial portion, reaching a plateau, and then decreasing with brighter intensities. The latter is supported by previous studies which showed that OPs and b-wave parameters as well as their luminance-response (LR) functions were highly covariant [17, 37–41]. For example, a correlation coefficient of 0.78 (p < 0.05) was previously reported between the b-wave amplitude and the sum of OPs (SOP) amplitude [39] and an even higher correlation coefficient of 0.98 (p < 0.05) was previously found between LR functions of the b-wave and OPs energy [17]. However, the nearly stimulus-independent %OPs value can be surprising to some given that, on visual inspection, waveforms evoked to progressively brighter stimuli appear to be gradually more oscillatory as exemplified in Figure 2. Nevertheless, DWT analysis suggests that more prominent and more numerous OPs on the ascending limb of the b-wave do not necessarily indicate a larger contribution of the OPs to the building of the ERG since the %OPs value was shown to be nearly stimulusindependent.

The above range of normal %OPs (56.6% to 61.6%) also demonstrates that, in normal subjects, the summed OPs energy contributes to more than half (OPs > 50%) of the total energy contained within the ERG waveform. Based on normal data included in previously published studies where OPs were extracted using bandpass filtering, we derived (see computation details in Table 3 caption) time domain %OPs (TD%OPs) values (reported in Table 3) as low as 26% [24] or as high as 112% [31]. As aforementioned, bandpass filtering can generate signal distortions such as phase lag, ringing artefacts, attenuation of OPs amplitude, and/or artefactual enhancement of OPs. The latter most probably accounts for the very large variation of the time domain (TD) derivation of the %OPs reported in Table 3. Intuitively, the "real" %OPs value should lie somewhere between the two extremes (i.e., close to the mean of all values). Of interest, the average normal TD%OPs derived from all previous studies (Table 3) was of $57.50\% \pm 30.01\%$, a value that is not significantly different (p > 0.05) from the %OP of the normal photopic ERG waveform (irrespective of stimulus intensity) that we quantified with the DWT $(59.10\% \pm 3.75\%$ as per Table 1). The latter would suggest that the %OPs computed with the DWT represents an accurate and highly reproducible (CV as low as 3.26% for the 0.9 log cd.s.m⁻² at Table 1) method to estimate the %OPs contribution to the building of the ERG.

Contrasting with the above findings obtained from normal subjects, data obtained from our patients did not present a constant %OPs value and showed a wide range of %OPs, some presenting with a relatively suppressed %OPs (such as 35.4%, CRVO ERG) and others with a relatively enhanced %OPs (such as 89.2%, end-stage RP ERG). Our analysis revealed that pathological ERGs presenting with OPs more prominent than normal (on visual inspection) disclosed a higher than normal %OPs, with some almost solely composed of OPs. However, ERGs from RP patients (see Figure 4) were sometimes of very low amplitude (and some with poor signal-to-noise ratio (SNR), e.g., Figure 4(b)) and, as such, one wonders if the %OPs value was repeatable within subjects. To investigate the latter, a representative patient was recorded twice to test for reproducibility (Figure 6). Despite the very low SNRs of 1.3 and 1.6 obtained on test and retest, respectively, the response morphology was found to be reproducible and so was the resulting %OPs (test: %OPs = 70.1%; retest: %OPs = 69.1%). The latter suggest that a repeatable measurement

of the %OPs can be obtained, even in low-SNR ERGs. To our knowledge, the findings reported herein demonstrate, for the first time, that in certain conditions the photopic ERG can be mostly (or solely) composed of high-frequency components, known as the OPs.

Interestingly, studies reporting on (slow sequence) multifocal ERGs of normal macaque monkeys revealed that responses obtained from the central retina were more oscillatory in nature and characterized with highly prominent high-frequency waves [6]. Similarly, focal macular ERG responses evoked from patients affected with RP also presented OPs that were relatively better preserved compared to the a- and b-waves, thus suggesting that macular responses are more oscillatory in nature [42], a finding also confirmed with focal macular ERGs obtained in a rabbit model of RP [43]. Given that most of our patients (as summarized in Table 2) that presented with highly prominent OPs had a very constricted visual field (which is a common finding in end stage of rod/cone dystrophies such as RP), the more oscillatory ERG responses that we found could find an explanation in the relatively better preserved central (macular) function. The above would therefore suggest that the macular region would contain a greater proportion of inner retinal cells which are suggested to be involved in OPs generation (i.e., amacrine, bipolar, horizontal, or ganglion cells) compared to retinal cells involved in the genesis of the bwave (i.e., bipolar and Müller cells). This claim would find support from previously published findings on retinal cell distribution in nonhuman primates [32-34]. Based on these studies, we computed (Table 4) a significantly greater bipolar-to-Müller cell ratio (BMR) at the fovea compared to more peripheral regions (i.e., 24, 40, and 65 degrees from fovea), thus supporting our claim.

In conclusion, normal subjects disclosed a relatively constant and highly reproducible %OPs values while patients presented with a significantly larger range of %OPs values. We postulated that, in some circumstances, a pathological ERG signal could be mostly (or solely) composed of OPs. Our analyses did indeed reveal that some ERGs were almost solely composed of OPs. Findings herein therefore support the hypothesis that, in certain conditions, the photopic ERG can be mostly composed of high-frequency components, known as the OPs. Furthermore, the significantly wider range of %OPs values measured in patients (with variable b-wave amplitudes) would suggest that one of the two

ERG components (b-wave or OPs) might be relatively more affected than the other and the DWT therefore represents a worthwhile approach to help in the segregation of pathological ERGs.

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10. Captions to Figures and Tables

Figure 1: Computation of the percentage of oscillatory potentials (%OPs) contributing to the ERG response. The DWT scalogram (A) is shown above the broadband ERG (B). Temporal sections of the scalogram that were taken into consideration when calculating the %OPs are outlined by the dotted gray lines in order to ease appreciation of the time-frequency region in question. The DWT descriptors (20b, 40b, 80ops and 160ops) that were used to compute the %OPs are identified on the scalogram. The inverse DWT (IDWT) reconstructions of the slow (a- and b-waves; indicated by the corresponding letters) and

fast (OPs; indicated with corresponding numbers) waves are shown as the black and red traces (panels C and D), respectively.

Figure 2: Normal ERGs (obtained at four different stimulus intensities) which show a relatively constant %OPs contribution (from A to D: 58.7%; 57.8%; 56.5%; 59.4%). The four examples are shown in increasing order of stimulus intensities from panels A to D. The DWT scalograms are shown at the top of each broadband ERGs. Sections of the scalogram that were not considered in the %OPs computation were shaded to facilitate comparison. The descriptors (20b, 40b, 80ops and 160ops) that were used to compute the %OPs are identified on each scalogram.

Figure 3: (A) %OP contribution to the photopic ERG response obtained from a normal subject and patients with Diabetic Retinopathy (DR) (B) and CRVO (C). All photopic ERG responses were evoked to the 0.64 log cd.s.m⁻² stimulus. Sections of the scalogram that were not considered in the %OPs computation were shaded to facilitate comparison. The descriptors (20b, 40b, 80ops and 160ops) that were used to compute the %OPs are localized on each scalogram. The %OPs is shown at the top of each DWT scalogram.

Figure 4: Pathological ERGs (obtained from four different end-stage RP patients) which show a significantly higher %OPs contribution. The four cases are shown in increasing order of %OPs from panels A to D. In each case, the DWT scalogram is shown on the top of the broadband ERGs. Temporal sections of the scalogram that were not considered in the %OPs computation were shaded to ease appreciation for the region in question. The descriptors (20b, 40b, 80ops and 160ops) that were used to compute the %OPs are shown on each scalogram.

Figure 5: (A) A control ERG (black tracing) is shown with the ERG follow-up of patients #16 (blue tracings) and 17 (red tracings), at first visit (12 and 17 years of age, respectively) and at second visit (24 and 19 years of age, respectively). The a-, b- and i-waves are indicated with their corresponding letters and OP2, OP3 and OP4 with their corresponding numbers. Calibration: horizontal: 50 ms, vertical: as shown to the right of each tracing. (B)

Corresponding DWT scalograms and associated %OPs. Descriptors (20b, 40b, 80ops and 160ops) that were used to compute the %OPs are localized on each scalogram. Note that in the scalogram corresponding to the 2nd visit of Brother 2 (bottom tracing), the 40b descriptor was identified using the value of the left rectangle (yellow) as the higher energy red rectangle was time-locked to the i-wave.

Figure 6: Representative test-retest repeatability of the %OPs. Tracings (top and bottom panels) were recorded twice in the same RP patient. The %OPs is shown at the top of each tracing.

Table 1: Normal variation of the %OPs as a function of the 21 stimulus intensities. Column 1: Stimulus intensities. Column 2: Mean %OPs values obtained at each stimulus intensity. Column 3: Coefficient of variation (CV) of the %OPs at each stimulus intensity. Column 4: Mean b-wave amplitude obtained at each stimulus intensity. Column 5: CV of the b-wave amplitude at each stimulus intensity. Range, mean (±1S.D.).

Table 2: Ophthalmological and electrophysiological findings of the end-stage patient cohort included in this study. Patient number, age, visual acuity, visual field findings, rodand cone-mediated ERG responses, diagnosis, %OPs values and associated Z-scores are each indicated in individual columns. The %OPs contributing to the cone ERG response and associated Z-scores are shown in the last two columns, respectively. The critical *z*-score was set to a value of 1.645 and therefore any value > 56.6 + 1.645*2.5 (i.e. 60.7) is considered to be significant. LP: light perception; Flat: extinguished ERG; RP: retinitis pigmentosa.

Table 3: Time domain (TD) derivation of the TD%OPs contribution to the photopic ERG from previous studies (column 1). Column 2: TD%OPs values are presented in increasing order and were computed as the sum of OPs (SOP) amplitude divided by the associated b-wave amplitude and multiplied by 100. Column 2: The type of filters that were used. N/A indicates unspecified filter type. Columns 4 and 5: ERG and OPs cutoff frequencies that

were used for bandpass filtering. Column 6: The attenuation that were used at the specified cutoff frequencies. N/A indicates unspecified attenuation.

Table 4: Ophthalmological Derivation of the Bipolar-to-Müller cell ratio at different

 retinal eccentricities from previous non-primate studies.
11. Figures and Tables

Figure 1:



Figure 2:



Figure 3:



Figure 4:



Figure 5:









%OPs = 55.1%



%OPs = 65.2%







(b)

Figure 6:



Table 1:

Intensity (log cd·s·m ⁻²)	Mean %OPs (%)	%OPs CV (%)	b-wave amplitude (μ V)	b-wave amplitude CV (%)
-2.23	60.27	12.64	1.23	33.74
-2.04	61.56	9.96	1.54	30.73
-1.81	61.27	8.41	1.93	26.79
-1.62	60.75	7.56	2.67	20.10
-1.41	58.19	7.15	3.70	20.28
-1.21	59.23	8.35	5.02	18.27
-1.0	60.08	9.13	10.86	17.60
-0.8	61.41	9.40	18.24	27.41
-0.62	60.47	6.66	28.61	21.25
-0.41	58.67	7.56	39.02	21.39
-0.23	56.60	6.12	51.26	23.68
-0.02	57.76	5.13	76.81	25.69
0.17	59.02	4.56	102.48	22.73
0.39	58.86	5.51	115.42	22.04
0.64	56.64	4.42	112.78	17.25
0.90	59.96	3.26	100.49	18.36
1.40	59.89	3.68	89.71	20.87
1.63	61.38	5.98	57.92	18.55
2.15	60.19	6.61	47.64	20.92
2.39	59.56	5.23	46.35	17.96
2.64	56.99	6.35	49.35	24.36
Range	56.6-61.6	3.3-12.6	1.2-115.4	17.3-33.7
Mean ± SD	59.10 ± 3.75	6.82 ± 2.29	45.86 ± 40.10	22.48 ± 4.13
Total CV	6.34		87.37	

Table 2:

Patient	Age	Visual acuity	Visual field	Rod ERG	Cone ERG	Diagnosis	%OPs	Z-scores
1	62	20/50	<20°	Flat	<10%	RP	61.1	1.78
2	61	20/400	<10°	Flat	<5%	RP	75.4	7.46
3	51	20/200	*	<2%	<10%	RP	78.4	8.67
4	9	20/20	<15°	Flat	<10%	RP	89.2	13.14
5	44	20/100	<10°	Flat	<10%	RP	82.0	10.30
6	35	LP	<10°	Flat	<2%	RP	86.5	11.92
7	75	20/400	<10°	Flat	<2%	RP	74.9	7.46
8	6	20/30	N/A	Flat	<5%	RP	73.4	6.65
9	35	20/20	N/A	Flat	<5%	RP	67.1	4.21
10	73	20/400	<10°	Flat	<5%	RP	78.7	9.08
11	39	20/20	<15°	<1%	<10%	RP	64.3	3.00
12	51	20/50	<15°	Flat	<10%	RP	68.6	5.02
13	44	20/20	t	Flat	<10%	RP	65.4	3.40
14	58	20/60	<10°	Flat	<5%	RP	78.5	8.67
15	53	20/100	<10°	Flat	<10%	RP	71.8	6.24
16	24	20/25	< 20°‡	<5%	<15%	Choroideremia	65.2	3.40
17	19	20/30	< 15° [‡]	<5%	<10%	Choroideremia	79.9	9.49

*Presence of an annular scotoma

 † Large temporal scotoma with isopter IVe and <10% with isopter IIe ‡ Isopter IIe.

Table 3:

Papers	TD%OPs (%)	Filter type	ERG cutoff (Hz)	OPs cutoff (Hz)	Attenuation (dB)
Miyake, 1990 [24]	26	N/A	5.3-100	100-300	N/A
Lachapelle et al., 1993 [25]	28	N/A	1-1000	100-1000	6
Peachey et al., 1991 [26]	36	N/A	1-1000	100-1000	3
Kizawa et al., 2006 [7]	38	N/A	0.5-1000	100-1000	N/A
Lachapelle et al., 1998 [27]	44	N/A	1-1000	100-1000	6
Holopigian et al., 1992 [28]	48	N/A	1-300	100-1000	N/A
Rufiange et al., 2005 [22]	69	N/A	0.3-500	75-500	6
Fortune et al., 2004 [29]	75	Blackman filter	0.3-3000	70-280	3
Kergoat et al., 2001 [30]	99	N/A	1-1500	50-1500	N/A
Peachey et al., 1987 [31]	112	N/A	1–1000	100-1000	3

Table 4:

Eccentricity	Cones density (cells/mm ²)*	Cone bipolar cells density (cells/mm ²) [†]	Müller cells density (cells/mm ²) [‡]	Bipolar-to- Müller cell ratio
Fovea	220,000	244444	30,000	8.15
24 deg.	35,000	4730	25,000	0.19
40 deg.	22,000	4889	20,000	0.24
65 deg.	17,000	1809	15,000	0.12

*Adapted from Wilder et al. [32].

[†]Adapted from Chan et al. [33]. [‡]Adapted from Distler and Dreher [34].

CHAPTER VII:

GENERAL DISCUSSION AND CONCLUSION

1. Preamble

To date, more than 150 years after its discovery, the ERG remains the most objective tool to assess retinal function and continues to be broadly used to validate the diagnosis and monitor the disease progression of a variety of retinal disorders. However, while major improvements were achieved in ERG recording technologies, the interpretation and the clinical applications of the ERG remain limited as its analysis is still confined to peak time and amplitude measurements. The latter restricts the categorization of the ERG to two main diagnostic classes: 1-reduced ERGs with normal peak time or 2reduced ERGs with delayed peak time (although ERGs can also be of normal or increased amplitude with or without delayed peak time). This research project demonstrates that the traditional analysis approach is (obviously) not able to robustly describe ERG features (e.g. morphology, complexity, etc.), or dissociate between the ON and OFF components of the photopic ERG without the use of a specialized stimuli/protocols, and is unable to quantify low SNR responses accurately. In an attempt to improve the usefulness and the diagnostic power of the ERG, we set out to investigate the following question: "Can advanced analytical approaches (such as the FFT, CWT or DWT) extract additional useful diagnostic or physiological information from the photopic ERG that could overcome some of the limitations mentioned above?"

The main empirical findings of this research project were manuscript-specific and presented within the respective chapters of this PhD thesis. The five chapters revealed that:

1-Time-frequency analyses (CWT and DWT) are superior to time domain and frequency domain analyses for photopic ERG analyses, and the DWT appears to be the best time-frequency approach, (Chapter II: Manuscript #1);

2-DWT descriptors can identify reproducible, physiologically meaningful, and diagnostically relevant features of the ERG over a broad range of signal amplitudes and morphologies and are differently/specifically affected in certain diseases (Chapter III: Manuscript #2).

3-Compared to the traditional time domain measures of the ERG, the DWT permits earlier detection of ERG anomalies, allows better ERG-based segregation of degenerative retinopathies and improves the monitoring of attenuated responses (Chapter IV: Manuscript #3).

4-The DWT can be used to accurately quantify the ON- and OFF-pathway contribution to the clinical short-flash ERG (Chapter V: Manuscript #4).

5-The DWT can be used to quantify the oscillatory potentials of normal and pathological ERGs without altering the signal (as done with the filtering) (Chapter VI: Manuscript #5).

Taken together, the five manuscripts (Chapters II to VI) included in this thesis demonstrate that advanced methods and, more specifically the discrete wavelet transform, reveal useful diagnostic features and highlights physiological information, that overcome several limitations of the traditional time domain measurements, suggesting that the DWT is a better method to analyse the photopic ERG compared to TD.

2. Beyond time domain measurements

Prior to this project, time-frequency analyses obtained using the wavelet transform had already been well documented and previously used to extract additional information from several biopotentials. Not only in the medical field, where they brought additional sensitivity and specificity to the detection and classification achieved with different biomedical tests (Van Drongelen, 2007), but also in finance, earth sciences, and physics, where they brought several insights about the world we live in (Addison, 2002; Mallat 2009). These small waves have helped understand and predict earthquake patterns (Gurley and Kareem, 1999), or extract meaningful information from gravitational waves (Abbott et al., 2016), thus bringing a better understanding of our universe and its creation. In this section, I will summarize the emerging role of the wavelet transform in the analysis of two biopotentials, namely, the (ECG) well-known electrocardiogram and the electroencephalogram (EEG), where it has been extensively used.

2.1 Applications of the wavelet transform to the ECG

The ECG (Einthoven, 1911) is a spontaneous biopotential which can be recorded virtually anywhere on the surface of the body. In contrast to the ERG, the ECG does not need a stimulus; the electrical activity is produced as a result of the natural contraction of the heart muscle (which generates the electrical current). Over its cycle, a heartbeat produces the components of the ECG, namely the P, Q, R, S and T waves, and each of them is associated with a different phase of the heart contraction (Addison, 2005). More specifically, the P-wave is associated with the contraction of the atria, the QRS complex with the contraction of the ventricles, and the T-wave represents their return to their resting potential (repolarization). Components can be measured in amplitude and timing (e.g. latency between two consecutive R-waves, also termed the RR interval), but interestingly, the analysis of the ECG morphology, using the wavelet transform, was also proven useful to extract meaningful information and has lead to several clinical applications, such as improved classification and automated diagnosis (Meste et al., 1994; Wiklund et al., 1997; Thurner et al., 1998; Al-Fahoum and Howitt, 1999; Ivanov et al., 1999; Yi et al., 2000; Chen, 2002; Duverney et al., 2002; Pichot et al., 2002; Toledo et al 2003; Nyander et al., 2004).

For example, the early work of Meste et al. (1994) used local wavelet maxima to detect and quantify late ventricular potentials of the ECG in the time-frequency scalogram and to assess the wavelet-based temporal and spectral variability in the ECG of patients with or without arrhythmia/tachycardia. They achieved a wavelet-based classification with 93% specificity (i.e. the percentage of healthy subjects who were correctly identified as not having the condition) and 85% sensitivity (i.e. the proportion of patients who were correctly identified as having the arrhythmia/tachycardia); which, by comparison, was more accurate than traditional methods, such as the time domain and FFT (Meste et al., 1994). Furthermore, Thurner et al. (1998) found that the standard deviations of the wavelet coefficients (at different frequency bands) can differentiate between normal subjects and patients with congestive heart failure with a 100% accuracy, compared to 80% using traditional inter-peak intervals. Similarly, Al-Fahoum and Howitt (1999) used an artificial neural network to process the wavelet transform data of ECGs obtained from arrhythmic patients (e.g. with either ventricular fibrillation or ventricular tachycardia). They also

reported a 100% correct classification for both groups, with better-segregated features, compared to other approaches (Al-Fahoum and Howitt, 1999). In another study, Yi et al., 2000 evaluated the diagnostic power of the wavelet transform in patients with idiopathic dilated cardiomyopathy and found that the wavelet transform was superior to the traditional time domain approach to detect patients at high-risk of clinical worsening. Of interest, some groups also studied the ratio between the energy of low-frequency (LF) and highfrequency (HF) wavelet coefficients. For example, Chen (2002) found that the LF/HF ratio was increased before the onset of ventricular tachycardia; a subtle feature that was unseen with the traditional TD approach. The LF/HF ratio was also later found to be associated with the perfusion of the myocardial muscle (Toledo et al. 2003). Interestingly, Duverney et al. (2002) also used wavelet-based fractal analyses to train an artificial neural network and achieved 92.6% specificity and 96.1% sensitivity for discriminating atrial fibrillation episodes from normal rhythm. Moreover, by studying wavelet-based measures of signal complexity (e.g. reminiscent of the Hölder exponent), Nyander et al. (2004) were also able to characterize the ON and OFF switching of the autonomic nervous system, thus demonstrating that the wavelet transform can reveal useful and subtle physiological information that is not readily available in the TD.

2.2 Applications of the wavelet transform to the EEG

The EEG (Berger, 1929; for an English translation of this paper, see Gloor, 1969) is another well-known biopotential often recorded using multiple electrodes pasted on the scalp. This signal objectively reflects electrical activity changes in the brain and has several clinical applications. To name a few, it can be used to identify and localize epileptic seizures, monitor alertness and depth of anesthesia/coma and can also localize brain damage following head trauma, strokes or tumors (Raj et al., 2012). Of note, the EEG can also be recorded using external stimuli (i.e. event-related potentials: ERP). Averaging of multiple stimulus-evoked responses (e.g. sensory, cognitive, or motor events) reveals several ERP components (e.g. N100, P300, etc.), which can then be analysed (Thakor et al., 1993). ERPs are widely used for the investigation of auditory, somatosensory and visual responses and can be utilized for the diagnosis of various neurological conditions (Raj et al., 2012). Of note, most of the wavelet studies conducted on biopotentials have been

published in the EEG and ERP fields, where wavelets were shown to be useful to derive relevant additional information from the signal and were shown to be superior to the traditional time domain analysis of the raw EEG (Thakor et al., 1993; Demiralp and Ademoglu, 2001; Akin, 2002; Ocak, 2009; Solhjoo et al., 2005; Lemm et al., 2004; Raj et al., 2012; Faust et al., 2015).

More specifically, several groups have successfully used wavelet transform analyses to extract meaningful information from the EEG of epileptic patients and improved the diagnostic power while leading to a better understanding of the disease process (Akin, 2002; Ocak, 2009; Faust et al., 2015). For instance, in comparing advanced analytical approaches, Akin (2002) showed that the FFT (which is commonly used in EEG analyses) is not able to resolve local epileptic spikes in long EEG tracings and that the FFT measures are sensitive to the length (i.e. duration) of the signal. He demonstrated that combining both time and frequency information with the wavelet transform was bettersuited and more sensitive than the FFT and raw EEG time series alone to isolate epileptic discharges in EEG tracings (Akin, 2002). Using DWT-based measures of signal complexity (similar to those obtained with the Hölder exponent), Ocak (2009) found significant differences between the EEG of epileptic and normal subjects and demonstrated that the DWT could detect seizures with nearly 100% accuracy, compared to 73% when relying solely on raw time domain EEG measures. Moreover, Faust et al. (2015) recently conducted an extensive review of more than 60 wavelet-based EEG studies in epilepsy. Looking through this fairly abundant literature, Faust and colleagues felt that the consensus among authors was that, compared to traditional EEG analysis approaches, the wavelet approach was more accurate in detecting and classifying epileptic spikes, and hence, to diagnose epilepsy. Based on their review, they claimed that it is now well established that the wavelet transform is the best method for EEG-based seizure detection and epilepsy diagnosis and that, so far, no other signal analysis methods can extract the epileptic EEG features more accurately and comprehensively (Faust et al., 2015).

Similarly, the emerging role of wavelet approaches in the fields of auditory and somatosensory ERP has also led to several pertinent clinical applications. For example, as early as 1993, Thakor et al. demonstrated that the wavelet decomposition was better than

either time domain or FFT measures to characterize and rapidly detect multiple changes in the morphology of somatosensory ERPs following neurologic injury (e.g. hypoxic injury: HI). More specifically, they found that two wavelet features had an important diagnostic value; the energy of the high-frequency components of the ERP (i.e. quantified using the DWT) displayed an early and a more rapid decline in response to HI (compared to time domain or FFT), while the energy of the low-frequency DWT components displayed a quicker recovery with reoxygenation (Thakor et al., 1993). Likewise, Demiralp and Ademoglu (2001) conducted an extensive characterization of ERP signal morphologies using the wavelet transform. They demonstrated that the DWT could segment the various waves of the ERP (e.g. the N100 and P300 waves), in multiple functional sub-components and be used to assess and reveal meaningful processes that are unseen in the raw ERP signal when solely considering the waves amplitude or timing (Demiralp and Ademoglu, 2001). Of note, Quiroga and Garcia (2003) also demonstrated that DWT-denoising, an approach that selectively reconstructs the most important DWT coefficients within precise frequency bands and time windows, could improve the quantification of noisecontaminated ERPs.

2.3 The rise of a wavelet revolution in ECG and EEG

To complement the above summary of wavelet applications in the ECG and EEG, an advanced search on PubMed reveals that, since 1992, 704 wavelet applications were published in the ECG (280 papers) and EEG (424 papers) fields alone, where nearly twice as many studies used the DWT compared to the CWT. While this number undeniably represents a rough estimate (given that the search undoubtedly missed out papers and wrongfully included some), it nonetheless clearly demonstrates that the WT has been extensively applied to these well-known biopotentials. In both areas, the wavelet transforms have emerged as the best analysis tool according to several researchers (Thakor et al., 1993; Meste et al., 1994; Wiklund et al., 1997; Thurner et al., 1998; Al-Fahoum and Howitt, 1999; Ivanov et al., 1999; Yi et al., 2000; Demiralp and Ademoglu, 2001; Akin, 2002; Chen, 2002; Duverney et al., 2002; Pichot et al., 2009; Raj et al., 2012; Faust et al., 2004; Nyander et al., 2004; Solhjoo et al., 2005; Ocak, 2009; Raj et al., 2012; Faust et al., 2015). The ability of the WT to decompose a signal into pertinent components and to

derive relevant features, which look at the signal from a different angle than the traditional time domain measurements, has led to hundreds of clinical applications which, according to the above summary, seem to surpass those based on traditional time domain or frequency domain (e.g. FFT) analysis in many cases.

However, the hundreds of wavelet papers that were published in these fields since 1992 still represent a very small portion of the complete ECG and EEG literature published since 1992. As a result, one wonders why, relatively speaking, so few wavelet papers were published in these fields so far, especially if they are quite widely recognized as being the best method. Is it the complexity of the method or its interpretation that discourages some? Is it the reluctance of certain conservative physicians regarding novel techniques? Is it simply the unavailability of customized wavelet software? I prefer to believe that with great progress comes great delays. Indeed, it takes time to globalize the applications of newer and better technologies. Perhaps, even in a world at the edge of potentially disastrous climate changes, the worldwide adoption of eco-friendlier technologies is excessively slow, even if it is already available. Indeed, it is not because Tesla (Palo Alto, California, United States) develops revolutionary electric cars and exceedingly efficient solar panels and batteries, that they will soon replace the fossil fuel industry. In the same order of idea, I am confident that, in the next 20 years or so, we will progressively see the rise of the wavelet transform applications, emerging as the gold standard for all biopotential analyses and in the next sections I will discuss how this could be done for the ERG.

3. The wavelet transforms in the ERG world

Just like the ECG, EEG, and ERP, the ERG is also a biopotential. However, in contrast to the above-discussed fields, where an abundant, and exponentially growing, wavelet literature already exists, ERG analyses performed in the time-frequency domain have only been very occasionally reported and hence, the wavelet revolution has been much slower in the ERG field (i.e. combining all types of ERG signals; e.g. flicker ERG, pattern ERG, multifocal ERG and full-field ERG). Of note as well, for the ERG, nearly twice as many papers (when excluding our 5 papers) employ CWT, compared to the DWT. More specifically, to our knowledge, a mere 12 research articles were conducted using the CWT (Barraco et al., 2007, 2010, 2011a, 2011b, 2014; Dimopoulos et al., 2014; Forte et

al., 2008; Miguel-Jiménez et al., 2015; Nair and Joseph, 2014; Penkala 2005, 2007, 2010), while only 7 wavelet studies were conducted using the DWT (Miguel-Jiménez et al., 2008, 2010, 2011; Nair and Joseph, 2014; Rogala et al., 2005; Varadharajan et al. 2000, 2007). The latter contrast with the ECG and EEG fields where twice as many scientific studies were performed with the DWT, and where an additional 685 papers were published (i.e. according to the abovementioned PubMed search). Of course, it is indeed probable that certain properties of the ECG and EEG favored their development and their greater number of applications. For example, the ECG signal is much slower than the ERG, the living heart is more easily visualized (especially in the early days of electrophysiology), and the ECG commonly recorded using multiple electrode derivations, thus allowing localization of the ECG potentials. As a result, each wave of the ECG was rapidly associated with a single phase of the heart muscle contraction and relaxation (through correlations with imagery and various clinical testing). This level of knowledge remains to be reached with the ERG which is much more complicated to correlates with neuronal function. Clearly, much less wavelet work has been conducted in the field of electroretinography at large, and below, I discuss previous wavelet studies that contributed to the field prior to my research project along with their limitations.

In particular, the pioneering work of Varadharajan and colleagues (2000) made use of the DWT decomposition to analyse scotopic ERGs in normal subjects and patients affected with Duchenne macular dystrophy and significant differences were found in all frequency bands. While this study nicely provided the first proof-of-concept that the DWT could be useful to segregate patients from healthy subjects, they did not compare their outcomes to traditional amplitude or peak-time measurements, which would most probably have revealed statistical differences as well; given that the pathological ERG responses were attenuated (Varadharajan and colleagues, 2000). Interestingly, they also specified that the different DWT frequency bands were independent of each other and suggested that they were representing independent processes, but unfortunately, these claims were not supported by any of their findings (Varadharajan and colleagues, 2000). In another type of ERG signal (the pattern ERG: PERG), Rogala and Brykalski (2005) used the DWT to compare PERG responses obtained from glaucoma patients and normal subjects. When used as input variables to a linear classifier (i.e. an algorithm that automatically finds the best linear combinations of input variables to obtain the best separability between two classes), the DWT features were shown to be superior to the traditional TD measures in classifying normal and pathological PERG waveforms (Rogala and Brykalski, 2005). More specifically, the misclassification rate was of 60% for traditional time domain parameters, while that obtained with the DWT was of 34% which, although far from perfection, is still much better than TD (Rogala and Brykalski, 2005). In another type of ERG signal (the multifocal ERG: mfERG), Miguel-Jiménez et al. (2011) used the DWT to compare mfERGs obtained from normal subjects and patients affected by glaucoma. While this study clearly showed that the DWT approach was more reliable at detecting changes in glaucoma patients, compared to traditional Humphrey visual field tests, it did not include a comparison to traditional amplitude and peak-time measurements of the mfERG (Miguel-Jiménez et al., 2011). Moreover, in a study on the full-field ERG, Barraco and colleagues (2011a) showed that the scotopic a-wave of normal subjects was composed of three main frequency components, namely 20, 140 and 180 Hz, and that the higher frequency components were significantly reduced and delayed in achromats. However, this study also did not include any comparison to the traditional amplitude and peak time measurements (Barraco and colleagues, 2011a), and like all studies conducted by this group, limited the analysis of the ERG to a single ERG wave: the scotopic a-wave. Furthermore, in all their studies, they claimed that the high-frequency components that they identified pertained to the a-wave (Barraco et al., 2007, 2010, 2011a, 2011b, 2014). In fact, I believe that both high-frequency components that they found pertained to the OPs as they were peaking between 19.1 to 27.2 ms, which correspond to the timing of early OPs seen on the rising phase of the scotopic b-wave at the stimulus intensities that they used (Barraco et al., 2007, 2010, 2011a, 2011b, 2014). Also suggestive of the latter wrong association, the frequency range of scotopic OPs was previously found to range between 130 and 200 Hz (Poppele and Maffei 1967; Breslin and Parker 1973; Dimopoulos et al., 2014). More recently, Nair and Joseph (2014) applied the DWT to the full-field ERG and found significant differences between the maximal wavelet coefficients (the coefficient of highest energy) of patients with cone-rod dystrophy and controls. Interestingly, this study was the first study to apply the DWT on the full-field cone ERG (besides our studies), but unfortunately, they only explored a single DWT coefficient (i.e. the maximal component) and, as such, it was not possible to assess if some components were more specifically affected than others in a given group (Nair and Joseph, 2014). Furthermore, similar to other studies, they did not formally compare their DWT outcomes to traditional analysis (a- and b-wave amplitudes and peak times), which was obviously reduced and delayed in the cone-rod dystrophy group (Nair and Joseph, 2014).

4. Pushing the boundaries of wavelet-based ERG knowledge

The above studies of the ERG clearly suggested that the analysis of normal and pathological ERGs with the wavelet transform can expose subtle changes that could lead to significant diagnostic improvements and provided a preliminary base for my own research. Nevertheless, the applications of the DWT to the ERG remained very restricted, and several important limitations of previous studies remained to be addressed. While the above sections discussed where we were before this PhD project and what was already done in terms of wavelet research in the ERG field, the following sections present and contrast our novel contributions (summarizing where we are now) to previous knowledge and reveal what main research gaps were addressed with our empirical findings.

4.1 Filling the research gap in DWT analyses of photopic ERG

As shown above, previous DWT-based ERG studies were almost exclusively limited to the analysis of the multifocal ERG (Miguel-Jiménez et al., 2008, 2010, 2011), pattern ERG (Rogala et al., 2005) or full-field scotopic ERG (Varadharajan et al. 2000, 2007). In fact, to our knowledge, only one study investigated the use of the DWT analysis to the full-field photopic ERG signal but, as aforementioned, was limited to a single DWT descriptor and did not include comparisons to the traditional time domain analysis (Nair and Joseph, 2014). Considering that the full-field ERG signal is by far the most widely used clinical ERG, with the most cited ISCEV standard (i.e. McCulloch et al., 2015) in the visual electrophysiology field at large, the fact that only two wavelet studies (one on the scotopic, one on the photopic) were previously applied to the full-field ERG is quite puzzling.

Of interest, our project was exclusively limited to the full-field photopic ERG waveform, and hence, our findings certainly contributed to fill the above mentioned

research gap of DWT-based photopic ERG analyses and provided new applications, as evidenced with our 5 research articles (Gauvin et al., 2014, 2015, 2016a, 2016b, 2016c). Our findings allowed us to better understand the photopic ERG signal and the physiological processes that are modulated following different stimulus manipulation (intensity vs. duration). Our studies were also the first to identify specific DWT features that define selected retinopathies (e.g. CSNB, CPCPA, RP, DR, CRVO, CHM). In the ERG field, our approach is also the first to characterize all components of the photopic ERG using the DWT scalogram, with well-defined descriptors for the a-wave, b-wave, OPs, as well as ERG morphology and complexity.

4.2 Comparing the DWT to the traditional time domain

Moreover, of all previous wavelet-based studies of the ERG, only Rogala and colleagues (2005) thoroughly compared the analysis performances of the DWT approach against the traditional time domain features (of the PERG in this case). Indeed, in nearly all ERG papers, the investigators only reported their wavelet findings without systematically comparing them with the traditional time domain approach (Barraco et al., 2007, 2010, 2011a, 2011b, 2014; Forte et al., 2008; Penkala 2005, 2007, 2010; Miguel-Jiménez et al., 2008, 2010, 2011, 2015; Nair and Joseph, 2014; Varadharajan et al. 2000, 2007). In contrast, performance comparisons of wavelet vs. traditional measurements were extensively performed in several ECG and EEG studies (Thakor et al., 1993; Meste et al., 1994; Wiklund et al., 1997; Thurner et al., 1998; Al-Fahoum and Howitt, 1999; Ivanov et al., 1999; Yi et al., 2000; Demiralp and Ademoglu, 2001; Akin, 2002; Chen, 2002; Duverney et al., 2002; Pichot et al., 2002; Toledo et al 2003; Lemm et al., 2004; Nyander et al., 2004; Solhjoo et al., 2005; Ocak, 2009; Raj et al., 2012; Faust et al., 2015). Obviously, such comparisons are an important step in determining the usefulness and the added value of a new approach (or its applications), and the latter most probably contributed to improve the popularity of the wavelet transform among authors in these vast areas of research. Clearly, more studies and more methodological comparisons were needed to determine if the wavelet approaches were superior to the traditional ERG measurements in terms of accuracy, early diagnostic and/or classification.

In contrast to previous papers, our studies systematically compared our DWT descriptors to the traditional a- and b-wave measurements of the ERG; comparing for the first time their reproducibility (e.g. coefficient of variation), sensitivity (e.g. to detect an anomaly at disease onset), and specificity (e.g. to distinguish different ERG responses). Similar to what was reported for the pattern ERG (Rogala et al., 2005; the only other study that systematically compared both methods in the ERG field), we also found that the DWT descriptors were superior (in reproducibility, sensitivity, and specificity) to the time domain (Gauvin et al., 2014, 2015, 2016a). We were also the first to extend our comparisons to two other advanced analysis approaches (Gauvin et al., 2014). More specifically, we reported the first comparative study of advanced analysis methods (i.e. frequency and time-frequency analyses) in which we compared the classical TD measurements of the photopic ERG to measurements obtained with the FFT, CWT and DWT in their usefulness and appropriateness to study the photopic ERG signal (Gauvin et al., 2014). With this report, we demonstrated that the DWT was the best way to look at the frequency components of the ERG, compared to the CWT, that did not provide as much easily accessible information/descriptors, and compared to the FFT, that does not have any temporal information and which lead to artifactual interpretations. Of course, timefrequency components can also be identified with the CWT (Barraco et al., 2007, Dimopoulos et al., 2014; Forte et al., 2008; Nair and Joseph, 2014; Penkala 2010). However, we have shown that, in the CWT scalograms, the wavelet coefficients are highly locally-correlated and commonly form large equipotential clusters (i.e. cluster in which all coefficients have the same energy values) that mask some ERG components; such as those of the a-wave (Gauvin et al., 2014). We have shown that the latter bring incertitude to identify the time-frequency coordinates (i.e. ill-posed coordinates) of the ERG components and thus complicate the extraction of robust, reproducible descriptors from the CWT scalogram.

We also compared, for the first time, the DWT and TD analyses in the application of early detection of retinal degeneration (Gauvin et al., 2016a). In a patient that initially presented with a normal retina that progressively degenerated, the DWT allowed a significantly faster detection of photopic ERG changes; several years before the traditional TD measurements, and long before all other ophthalmological exams (visual acuity, visual field, retinal fundus, etc.). This case also allowed us, for the first time to our knowledge, to witness the earliest manifestations of the RP disease process and to see how it progresses to yield the full clinical picture of RP. We therefore demonstrated that the DWT can be used to more rapidly detect a retinal anomaly. The latter complements previous findings in ECG/EEG which have also shown that the DWT allows earlier detections (Thakor et al., 1993; Polikar et al., 2002; Geman and Zamfir, 2012; Rogala et al., 2005).

Lastly, at the end-stage of severe retinal degenerations (such as RP), the ERGs are usually characterized by low-amplitude and low-SNR responses (Bock et al., 1998; Gur and Zeevi, 1980; Rispoli et al., 1994). Of interest, we compared the DWT and the TD in their ability to monitor such advanced degenerative retinopathies. We have found that the DWT allowed significantly longer photopic ERG follow-up of disease progression, compared to TD measurements, which were corrupted by noise (low-SNR responses), or were simply not measurable, in the worst cases, when the a- or b-wave were indistinguishable from noise (Gauvin et al., 2014, 2016a). Again, these results agreed, with previous findings of other types of biopotentials, which also suggest that more accurate quantification of noisy signals can be done with the DWT compared to the TD (Quiroga and Garcia, 2003).

Overall, our comparisons of TD and DWT findings agree with previous wavelet reports in ECG/EEG which also suggest that the DWT is the best approach for biopotential analyses (Thakor et al., 1993; Meste et al., 1994; Wiklund et al., 1997; Thurner et al., 1998; Al-Fahoum and Howitt, 1999; Ivanov et al., 1999; Yi et al., 2000; Demiralp and Ademoglu, 2001; Akin, 2002; Chen, 2002; Duverney et al., 2002; Pichot et al., 2002; Toledo et al 2003; Quiroga and Garcia, 2003; Lemm et al., 2004; Nyander et al., 2004; Solhjoo et al., 2005; Ocak, 2009; Raj et al., 2012; Faust et al., 2015).

4.3 Characterizing the morphology of the ERG with DWT decomposition

As indicated above, the analyses of subtle changes in signal morphology may be achieved at different frequency bands using the wavelet transform and have brought several novel applications in the ECG (Meste et al., 1994; Wiklund et al., 1997; Ivanov et al., 1999; Yi et al., 2000; Chen, 2002; Duverney et al., 2002; Pichot et al., 2002; Toledo et al 2003; Nyander et al., 2004) and EEG fields (Thakor et al., 1993; Demiralp and Ademoglu, 2001; Akin, 2002; Ocak, 2009; Solhjoo et al., 2005; Lemm et al., 2004; Raj et al., 2012; Faust et al., 2015). In contrast, this potential powerful diagnostic feature of the ERG remains widely unexploited in clinics and basic research. Moreover, as shown throughout this thesis, morphologically distinct (and pathologically distinct) ERGs can have equal (or similar) TD measures and therefore may be inappropriately categorized in the same diagnostic class. For example, CPCPA and CSNB ERGs may present with indistinguishable traditional aand b-wave parameters, even if their morphology is strikingly different (Garon et al., 2014; Lachapelle et al., 1998; Gauvin et a., 2015). While the pathognomonic presentation of CPCPA and CSNB ERGs can be easily detected by visual inspections, the objective quantification and classification of more subtle, non-pathognomonic, morphological changes were never explored before. Therefore, our studies are, to our knowledge, the first to make use of the DWT to specifically derive morphology-sensitive descriptors that quantify several aspects of the ERG shape (Gauvin et al., 2014, 2015, 2016a, 2016b, 2016c).

For example, we introduced a new measure (i.e. the %OPs; derived from our DWT descriptors) to accurately quantify the relative contribution of the OPs to the ERG in an amplitude-independent manner (Gauvin et al., 2016c). The %OPs could be used to segregate more oscillatory responses from less oscillatory responses, thus offering an easy mean to segregate pathological ERGs based on their morphology (Gauvin et al., 2016c). Using this technique, we have also shown, for the first time, that in normal ERGs, the relative contribution of OPs to the ERG was almost constant, not stimulus-dependent and consequently little influenced by the absolute amplitude of the b-wave (Gauvin et al., 2016c). While it was known that DR and CRVO can be more specifically detrimental to the fast waves of the ERG (i.e. the OPs), we reported that, in other diseases (e.g. RP and CHM), the ERG waveforms could also have increased %OPs, suggesting, for the first time, that retinopathies can also be more detrimental to the slow frequency generators of the ERG (e.g. b-wave) (Gauvin et al., 2016c).

4.4 Determining the distinct origin of selected DWT descriptors

To our knowledge, no CWT or DWT study had previously aimed at identifying the physiological origin of the time-frequency components of the full-field ERG (or of any other types of ERGs). Of interest, we reported the first DWT analyses of ERGs obtained from patients affected with ON or OFF retinal pathway anomalies (Gauvin et al., 2015). Based on this data, we revealed, for the first time, that distinct time-frequency components can be more specifically associated with the function of the ON (20 Hz) and OFF (40 Hz) cone pathway (Gauvin et al., 2015). Likewise, we reported the first application of the wavelet transform to long-flash ERG analyses (Gauvin et al., 2016b) and, for the first time to our knowledge, we revealed the time-frequency composition of the b- and d-waves. The ON- and OFF-responses were mainly characterized with a 20 and 40 Hz component, respectively, thus confirming that the DWT allows for a more accurate determination of the ON (20 Hz) and OFF (40 Hz) retinal pathways in short- and long-flash ERGs (Gauvin et al., 2016b). Our findings are reminiscent of previous findings which also showed that the DWT could be used to decomposed a variety of biopotential waves in different physiological sub-components, such as the ON and OFF switching of the autonomic nervous system from ECG data (Nyander et al., 2004) or the decomposition of distinct and parallel neural events underlying the P300's wave of the ERP signal (Demiralp and Ademoglu, 2001). Of interest, using this capacity of the DWT to quantify the ON and OFF pathways more specifically, we also demonstrated, for the first time, that in most instances, the RP disease process could more severely impair the OFF retinal pathway (60% of cases), compared to ON pathway anomaly (10% of cases) or no preferential effect to either retinal pathway (30% of cases), thus offering a new method to further segregate degenerative retinopathies with the DWT (Gauvin et al., 2016a).

4.5 The choice of mother wavelet does matter

It seems from previous wavelet-based ERG studies that most investigators assumed that the wavelet transform needs to be conducted using a mother wavelet that bears some resemblance with the global shape of the signal of interest (Barraco et al., 2007, 2010, 2011a, 2011b, 2014; Dimopoulos et al., 2014; Forte et al., 2008; Penkala 2005, 2007, 2010; Nair and Joseph, 2014; Rogala et al., 2005; Varadharajan et al. 2000, 2007). This

widespread belief most probably came from wavelet applications such as data compression or signal detection (e.g. such as epileptic spike detection), where the wavelet needs to perfectly match the shape of the signal for better detection/compression (Mallat, 2009). For example, in their study of the scotopic a-wave, Barraco et al. (2011) used the Mexican Hat wavelet, which closely resembles the scotopic ERG waveform. Theoretically, if a wavelet function globally matches the entire waveform, then a single wavelet coefficient can be used to robustly detect and explain most of the signal (Mallat, 2009). The latter leads to a "sparse representation" of the signal where the maximal wavelet coefficient globally encompasses the information we are looking for (e.g. the signal). As a result, the signal can be fairly reconstructed using the single wavelet coefficient value, thus compressing the amount of information needed to represent most of the signal. However, to analyse all local morphological features of a signal (such as that of the a- and b-waves), then a compact wavelet (i.e. specialized in very local measurements, with high temporal resolution) must be chosen (Gauvin et al., 2015). Given that Barraco and colleagues (2011) chose a wavelet that matches the full ERG signal, they had to remove the b-wave from the signal (by cropping the ERG) prior to wavelet transform computation; otherwise, the identified maximal wavelet coefficients would have quantified the ERG signal more globally instead of specifically quantifying the a-wave. Similarly, in our early work, we had initially compared several wavelet functions, and when the wavelet shape perfectly matched the global signal, the luminance-response functions of the a-wave energy descriptors were corrupted by the b-wave and wrongly presented with a photopic hill-like shape.

Of interest, the most compact wavelet is by far the Haar wavelet (Mallat, 2009). It turns out that it is also the simplest wavelet function, and this is why we made use of this wavelet, for the first time in the ERG field, to quantify the local ERG features, such as the a-wave and b-waves, separately (Gauvin et al., 2014). Using the Haar wavelet, allowed us to more independently quantify the different components of the ERG signal and, as a result, the luminance-response functions of the a- and b-wave amplitude, which were previously described by us and others (Wali and Leguire, 1992; Garon et al., 2010, 2014; Hamilton et al., 2007; Kondo et al., 2000; Rufiange et al., 2002, 2003, 2005; Ueno et al., 2004).

In contrast, to quantify the ERG more globally, we have made use of the symmetric Daubechies wavelet (SDW) (Lina and Mayrand, 1995), which was never previously used in the ERG field [note that Rogala et al. (2005) and Varadharajan et al. (2000, 2007) used the regular Daubechies wavelet and not the SDW]. Given its morphology, the SDW set with two vanishing moments better encompass the entire ERG waveform. This wavelet is also blind to low-order polynomials that are nonspecific to the ERG waveform and permit a representation of the genuine ERG waveform and a more global measurement. Using DWT descriptors, we studied, for the first time, the luminance-dependence of the short-flash cone ERG components (Gauvin et al., 2015, 2016b). Luminance-response curves generated using selected DWT descriptors of the ERG revealed distinct luminance-dependence patterns, demonstrating, for the first time to our knowledge, that the stimulus luminance differently modulates the various time-frequency components of the ERG and thus its morphology (Gauvin et al., 2015).

5. Theoretical implications of the DWT for ERG analyses, diagnoses, and monitoring

As indicated in the above section, our project contributed to new knowledge and provided several missing scientific demonstrations that remained to be performed to confirm the usefulness of the DWT in the ERG field. Nevertheless, many additional wavelet studies will be needed for the ERG to reach the same diagnostic power than what is reported for the ECG and EEG. This section discusses important theoretical aspects of our discoveries which we believe will help improve the diagnostic power of the ERG and its usefulness in basic and clinical research.

Several retinopathies lead to alterations of the signal amplitude (with or without delay) and based on amplitude, a broad range of diseases (or stage of and/or phenotype of and or genotype of) can end up in the same ERG diagnostic classes even if the responses are completely different in shapes (Gauvin et al., 2015, 2016a). Similarly, when relying solely on the a- and b-wave amplitudes, some pathological ERGs could be wrongfully marked as being normal even when their morphologies suggest an abnormality, hence jeopardizing the detection of subtle ERG anomalies at disease onset (Gauvin et al., 2016a). We believe that the ERG has the potential to be a more powerful diagnostic tool, and findings reported in this thesis suggest that DWT analyses represent one the first step in

improving its diagnostic power. Indeed, it is evident from the above data that DWT descriptors can be differently and more specifically affected by a given disease process and, consequently, that they can be used to detect meaningful differences between various diseases or simply between ERG responses of different morphologies. Clearly, the DWT analysis can yield valuable information that could impact the interpretation and improve the classification of pathological ERGs.

This is well summarized in Figure 4 which shows pathological ERGs obtained from patients affected with retinal degenerations of different etiologies. In order to consider the differences in ERG composition that are solely due to changes in the waveform morphology (and not resulting from amplitude differences), prior to DWT computation all the pathological ERGs shown in Figure 4 were normalized (see amplifications factors on the right-hand sides of the tracings) to the mean ERG amplitude reached in normal subjects in response to the 0.64 log cd.s.m⁻² stimulus. From the traditional TD analyses, the pathological ERGs shown in Figure 4 belong to the following diagnostic categories: class #1- ERGs that are reduced in amplitude with normal timing (Figure 4A, tracings 2, 3 and 4) or, class #2- ERGs that are reduced in amplitude with delayed timing (Figure 4B, tracings 2, 3 and 4). However, their morphologies are also strikingly different (i.e. even if in the same category) and, as a result, several DWT descriptors did differ significantly, as evidenced by data and Z-score statistics reported in Table 3.

In all six examples, the Hölder coefficient was found to be significantly lower than normal, indicating that all six waveforms are considerably less complex than normal. Of interest, this parameter can thus be used to segregate responses based on their level of complexity [A2 most complex (z-score: -2.4) and A4 least complex (z-score: -9.76)]. Other waveform differences include an abnormal 40a descriptor in A2 and A3 only, an abnormal 20b descriptor in A4, B3 and B4 only, an abnormal 40b descriptor in A2 and B2 only, a normal 80ops descriptor in A4 and B4 only, a normal 160ops descriptor in A3 and A4 only and an abnormal delta-variance descriptor in A3 and A4 only. Of interest, the sign of the Z-scores, which indicates the direction of the abnormalities (i.e. negative: reduced parameters; or positive: increased parameters), can also be used to further discriminate the different ERG responses. For example, the 20a is reduced in A2, but increased in in A3 and the 40b is increased in B3, but reduced in A2.



Figure 4. Photopic ERGs evoked to a stimulus of 0.64 log $cd.s.m^{-2}$ in intensity and recorded from a normal subject (Panels A and B, tracings 1) and patients affected with retinal degenerations and presenting with either reduced ERG amplitudes with normal timing (Panel A, tracing 2, 3 and 4) or with reduced ERG amplitudes with delayed timing (Panel B, tracings 2, 3 and 4). In both panels, the red dashed line is time-locked to the normal b-wave (tracing 1) to distinguish between normal (panel A) and delayed (panel B) b-wave peak-times. ERG amplitudes were normalized (i.e. amplified) to that of control at the same intensity (as indicated by x3.16, x1.82, x4.94, etc. on the right-hand side of the waves).

Clearly, when analysed with the DWT descriptors, each of the six pathological ERGs of Figure 4 had a unique time-frequency domain signature and all were statistically separable from each other (as per different distribution of Z-score statistics). The data presented in Table 3 thus provide an interesting empirical demonstration and theoretical implication of how the DWT analysis could complement the traditional analysis of the ERG by offering an alternative quantification, which could better reflect the theoretically

unlimited ways by which retinal function (as reflected with the ERG) can be altered as a consequence of normal and pathological processes.

Table 3. DWT descriptors values for ERGs shown in Figure 4. Values between brackets indicate Z-score statistics (Bold: significant negative Z-score, p < 0.05; Bold + Italic: significant positive Z-score, p < 0.05). Control values are reported in the right column.

ERG tracing	A2	A3	A4	B2	В3	B4	$Control \pm SD$
Intensity	0.64	0.64	0.64	0.64	0.64	0.64	0.64
20a*	33.64 (-2.75)	188.72 <i>(7.07)</i>	144.85 (4.29)	165.94 (5.63)	123.71 (2.96)	190.1 <i>(7.17)</i>	77.08 ± 15.77
40a*	58.48 (-2.13)	166.14 (2.5)	95.13 (-0.55)	102.61 (-0.23)	123.02 (0.65)	122.5 (0.63)	107.96 ± 23.22
20b*	312.45 (1.49)	237.11 (-0.19)	371.69 (2.81)	228.43 (-0.39)	359.68 (2.55)	350.49 (2.34)	245.71 ± 44.69
40b*	153.12 (-2.69)	296.79 (1.05)	271.95 (0.41)	324.08 (1.77) †	208.16 (-1.26)†	240.87 (-0.41)†	256.45 ± 38.27
80ops*	70.58 (-1.75)	127.75 (2.27)	76.81 (-1.32)	137.21 (2.94)	45.23 (-3.54)	84.13 (-0.80)	95.50 ± 14.2
160ops*	27.25 (-3.15)	50.40 (-0.47)	43.93 (-1.22)	75.87 (2.48)	17.18 (-4.31)	34.96 (-2.25)	54.43 ± 8.64
Hölder*	1.06 (-2.4)	0.83 (-6.96)	0.69 (-9.76)	0.97 (-4.20)	0.79 (-7.80)	0.76 (-8.40)	1.18 ± 0.05
Delta-variance*	29.56 (0.13)	41.27 (1.98)	23.56 (-2.17)	31.39 (0.42)	22.00 (-1.07)	16.76 (-1.90)	28.76 ± 6.3
%OPs*	45.67 (-4.39)	57.17 (0.21)	42.87 (-5.51)	67.60 (4.38)	30.54 (-10.44)	44.61 (-4.81)	56.64 ± 2.5
40b-to-20b*	0.49 (-9.33)	1.25 (3.36)	0.73 (-5.31)	1.42 (6.15)	0.58 (-7.85)	0.69 (-6.05)	1.05 ± 0.06

* ERG b-wave amplitudes are normalized to the mean amplitude at 0.64 log cd.s.m⁻² before computation of DWT descriptors. † 40b descriptors located at delayed position.

Furthermore, significant changes can also be detected even if the amplitude of the pathological ERGs is normalized to the amplitude reached at the same intensity in normal subjects (approximately 110 μ V). In other words, from a b-wave amplitude point-of-view, all ERGs shown in Figure 4 would have been considered normal, although our descriptors reveal that their morphology is clearly not. Using selected amplitude-independent descriptors (those that lead to identical values with or without prior magnification), these changes were statistically pronounced and exposed very significant differences between these morphologically-distinct ERGs, as revealed by the very low or high z-scores of the reduced or increased %OPs in B3 (i.e. -10.44) and B2 (4.38), or of the reduced or increased 40b-to-20b ratio in A2 (-9.33) and B2 (6.15). This information can then be used to easily differentiate ERGs that have more or less prominent OPs or a more pronounced ON or OFF pathway anomalies.

The latter illustrate how our DWT descriptors can detect subtle ERG anomalies that lead to major statistical changes (with Z-scores as low as -10.44 and as high as 7.17) even when the TD measures (amplitudes and timing) are still normal (once normalized),

strengthening the concept that the DWT could be instrumental to more rapidly detect subtle photopic ERG anomalies (such as those presented in Gauvin et al., 2015 and Gauvin et al., 2016a). It is clear from Figure 4 and Table 3 that DWT descriptors can be differently affected by a given disease process and hence, that they could be used to further segregate ERGs (that would otherwise be assigned to the same diagnostic category) in multiple new diagnostic classes.

Nevertheless, it might appear unfair to say that the DWT leads to more diagnostic classes than time domain measures, because for certainty, if we measure more parameters (instead of solely looking the a- and b-wave parameters), we will necessarily have more potential diagnostic classes. Indeed, there are many other ways to extract more ERG features from the time domain signals. For example, instead of measuring the amplitude and peak time at the lowest trough (i.e. a-wave) and highest peak (i.e. b-wave) of the ERG (as per ISCEV guidelines), we could also measure the signal at 25, 50, 75% of the a-wave and b-wave amplitudes. We could assess the timing and amplitude of the i-wave or of the photopic negative response (PhNR), which were not studied herein. We could also measure the OPs in the TD, albeit this would necessitate bandpass filters, known to induce artifacts, and which are prone to over- or under-attenuation of the OPs. Nevertheless, OP measurements in the TD were previously successfully used to distinguish different retinal disorders (Lachapelle et al., 1998). Likewise, Fourier analysis (such as the FFT) could also have been used to distinguish between different ERG signals by determining the frequency of their fundamental components. Ratio between the b-wave and a-wave amplitude could also have been used to identify morphological differences between ERG responses (Jung et al., 2015). Moreover, although necessitating the recoding of several stimulus intensities, the cone ERG luminance-response function (i.e. the photopic hill) could also have been used to distinguish between ON or OFF retinal pathway anomaly (Garon et al., 2014). Lastly, in the most comprehensive case, we could even measure all amplitude values (at each time point) of the signal in the time domain, which could easily lead to several hundred features (depending on the number of data points and sampling rate). Clearly, there are other TD parameters that can be used to complement the traditional a- and bwaves measurements. Notwithstanding the above, I hope that results presented in this thesis convinced the reader that the DWT analysis significantly eases the process of thoroughly and efficiently quantifying the ERG, in all its subtlety, by optimally decomposing the response in its various time-frequency components and by offering an ideal scheme (the DWT scalogram) to derive descriptors and to perform advanced signal processing (such as the inverse DWT).

In the field of digital signal processing, a feature is, by definition, a unique characteristic, a descriptor, a component, or any measurable information that can be derived from a signal or segment of (Cvetkovic et al., 2007). Features are used to efficiently describe waveform patterns by solely compiling pertinent information. The set of all features used to describe a signal (or patterns of) is called the feature vector and can be used to decrease the data space required to represent a signal with very minimal loss of information (Mallat, 2009). The latter principles form the basis of the data compression theory and is also needed for optimal artificial intelligence applications (e.g. such as learning algorithms for automated classification). While having hundreds of signal features (and in theory an infinite number of features) might sound appealing to some, it has been shown that having so many features does not necessarily lead to more relevant information (Kwack and Choi, 2002). Furthermore, it is well known that the performance of automated classifiers worsens and slows down when too many irrelevant or redundant features are provided (Kordylewski et al., 2001; Kwack and Choi, 2002; Übeyli and Güler, 2005). Therefore, in order to make rapid and accurate automated segregation with classifiers, such as with artificial neural networks, one first needs to convert the complex patterns of a signal into simplified and relevant features, which become a condensed representation of the signal that only includes essential properties (Kordylewski et al., 2001; Kwack and Choi, 2002; Übeyli and Güler, 2005). Of interest, this is exactly what we did in this thesis; we extracted the complex patterns of the ERG signal into a simplified set of relevant DWT descriptors.

Briefly, we have shown that the DWT was able to extract different descriptors (20a, 20b, 40a, 40b, 80ops, 160ops, Hölder exponent, Delta-Variance, 40-to-20b and %OPs) of the ERG waveform and we have demonstrated (through inverse wavelet transform reconstructions) that selected descriptors were sufficient to represent the complete ERG waveform (Gauvin et al., 2015). We have shown that DWT descriptors were highly

reproducible and varied in a predictable fashion when looking at known ERG-modulation methods, such as the photopic hill. We have also demonstrated that the DWT descriptors can be extracted from very low-voltage and/or low-SNR ERGs, thus allowing for a more prolonged follow-up of disease progression (Gauvin et al., 2014, 2016a). Moreover, we have also shown that distinct descriptors were more specifically affected by different diseases, whereas other were not affected, suggesting that several diagnostic classes could be derived from these descriptors. Given all the above findings, our research suggests that our set of 10 novel descriptors will be an optimal feature vector for various classification algorithms (so-called classifiers).

In theory, our 10 descriptors could bring a minimum of 1024 (2¹⁰) new ERG diagnostic classes, thus leading to the creation of an extensive dictionary of classes. Obviously, not all 1024 permutations of our 10 DWT descriptors will allow the classification of specific retinopathies (or stage of), however, the fact that already a fraction of those combinations does so (as suggested by Figure 4 and Table 3 and by data presented in our papers), could trigger an exciting new era in ERG research.

6. Study limitations and direction for future research

Although our five studies contributed new knowledge in the ERG field, with promising future applications, it is also important to recognize the main limitations of our research. The DWT approach described in the present thesis has the potential to refine the ERG sensitivity and specificity to the point that, one day, we may be able to identify ERGs that will be characteristic of a given disease process, stage of disease, or specific genotype. However, before reaching this possible future, additional work will be needed to address some limitations of our studies, which are summarized below along with potential research directions.

Firstly, I believe that future studies could use our set of DWT descriptors as a feature vector to further evaluate their usefulness in advanced classification applications (e.g. artificial neural network and other classifiers and algorithms, etc.) where much more work remains to be done on the ERG. Given that it was the case in the ECG and EEG fields, I assume that classification systems could eventually lead to the creation of a robust

automated ERG-based evaluation of diseases/symptoms, which could result in more accurate diagnoses and prognoses for the affected patients. For certainty, an automated classification system is also expected to have positive impacts on patient management, especially now that clinical trials exploring several new avenues (e.g. replacement of photoreceptors, gene editing, etc.) for the treatment of retinal degenerations are now underway. Therefore, it is my opinion that the next logical step should be to compile DWT data from thousands of ERGs (obtained from normal subjects and patients affected with different retinal disorders) and to use a variety of classifiers to derive new classes and various ERG patterns (such as the six classes shown at Figure 4 and Table 3). These classes/patterns could then be correlated to other clinical descriptors such as clinical diagnosis, sex, age of patients, age at onset of symptoms, habits, use of drugs, subjective evaluation of severity, family history, fundus pictures, visual fields, visual acuity, color vision, etc. to derive a comprehensive clinical interpretation of these classes. It will also be interesting to examine possible correlations between the molecular defect in the retinal pathways and the new ERG diagnostic classes. Advanced genotyping offers the unique opportunity to test patients affected with a wide range of retinal degenerations such as: retinitis pigmentosa, cone-rod dystrophy, Leber congenital amaurosis, choroideremia and congenital stationary night blindness, to name a few, where already more than 300 genes have been mapped and more than 250 mutations been identify as causing retinal degeneration (Hafler, 2016). Indeed, in an era where genetic sequencing is exploding, it will be highly exciting to look if correlations exist between the DWT photopic ERG descriptors and the genetic mutations of given patients.

Based on the statistical significance (i.e. the Z-Scores, p-values) of different pathological changes observed from various TD and DWT descriptors, we can safely conclude that DWT descriptors are significantly more altered than traditional TD parameters in several retinal conditions, to the extent that in certain cases, selected DWT parameters were significantly affected even when the TD descriptors were still within normal limits (Gauvin et al., 2015, 2016a, 2016c). However, while these findings strongly suggest that the DWT descriptors can more rapidly detect retinal anomalies, additional DWT studies are warranted to confirm the earlier detection in patients at the very onset of their disease and presenting with marginally normal photopic ERGs (such as in the 30 years

follow-up of RP presented in Gauvin et al., 2016a). Given that this type of patient rarely presents in clinics, it would be interesting to rely on animal studies to further test this claim. For example, guinea pigs were previously shown to have a photopic ERG that perfectly mimics (in amplitude, timing and frequency domain) the human photopic ERG (Racine et al., 2005), and theoretically one could use well-established animal models of progressive retinal degeneration (e.g. such as N-methyl-N-nitrosourea (MNU)-induced retinopathy; Tsubura et al., 2010, or postnatal bright light and oxygen exposure to mimic outer and inner retinal diseases, respectively; Joly et al, 2006; Dorfman et al, 2008) with multiple ERG recording time points to see if the DWT can detect functional changes before the traditional TD parameters and further prove that the DWT descriptors can detect significant changes faster. Similarly, small cryogenic lesions (as per Casanova et al., 2002) could be created (e.g. in Guinea pigs or other rodents) in order to temporarily disable selected regions of the retina to mimic early focal (central or peripheral) retinal lesions and to determine if the DWT parameters are altered before the traditional parameters. Alternatively, small local laser lesions (as per Belokopytov et al., 2010) could also be created to imitate early retinal lesions, albeit in a less temporary manner. Clearly, the above approaches could be used to perform direct comparisons between the guinea pig's ERGs and those of humans to further confirm the earlier detection capability of the DWT.

Throughout our studies, we have found that the physiological origin on the 20Hz and 40Hz components of the b-wave (20b and 40b descriptor, respectively), were the ON-and OFF-pathways, respectively (Gauvin et al., 2015, 2016b). However, it would be highly interesting to confirm these findings following programmed interferences with the normal functioning of the retina using pharmacological blockage in animals, such as in the aforementioned Guinea Pig model of human photopic ERG (Racine et al., 2015). Indeed, it was previously shown that intravitreal injections of selective blockers of the ON or OFF retinal pathways could mimic human retinopathies (e.g. CSNB) known to result from the same functional anomalies (Racine et al., 2015). The latter would also help us to further determine the retinal malfunction (cellular of pathway) at the origin of any equivalent abnormal human photopic ERG or to identify the origin of other DWT descriptors that remains to be elucidated (20a, 40a, 80ops, 160ops, etc.). While our OPs descriptors (80ops and 160ops) do not, unlike band-pass filtering, permit the analysis of the photopic OPs
individually, our results did show that the two OP frequency bands (80 Hz and 160 Hz) can differ in their respective energy level (see Table 3 and Gauvin et al., 2015), suggesting that they could potentially quantify different features of the high-frequency components of the ERG. To achieve a better understanding of any of our DWT descriptors (and their origin), photopic ERGs of Guinea pigs should be analysed with the DWT following intravitreal injections of 2-amino-4 phophonobutyric acid (APB: retinal ON pathway blocker), Cis-2,3 piperidine dicarboxilic acid and Kynurenic acid (retinal OFF pathway blockers), glycine (known to selectively abolish the oscillatory potentials) or TTX (known to selectively abolish the ganglion cell activity) to investigate if different blockages affect different descriptors.

In this thesis, we elected to limit our analyses to the photopic ERG waveform but, of interest, our approach could be used to classify the scotopic ERG waveforms and its morphology. Nevertheless, given that the peak-time of the scotopic ERG varies significantly more (e.g. the scotopic b-wave implicit time ranges from 40 and 125 ms for the same range of stimulus intensities; as per Garon et al., 2010; Velten et al., 2001), it might be necessary to select an optimal range of intensities, and the choice of wavelet function and DWT descriptors might need some adjustments. Of interest, in the mixed rodcone ERG, the rod and cone responses are blended together. Given that the DWT excelled at separating the ON- and OFF-pathways of the photopic ERG, one wonders if it would also be possible to decompose the rod and cone response from the mixed rod-cone ERG. Perhaps, patients affected with a pure-rod anomaly or a pure-cone anomaly could be recorded in mixed rod-cone conditions, and their ERGs analysed using the DWT to extract their time-frequency contributions. Missing DWT components from the two models could indicate which components are associated with the cone and rod response, respectively. Theoretically, maybe that one day the DWT will be able to extract all meaningful information from this type of mixed ERG response and derive a full retinal function pedigree using a single ERG. Similarly, in this thesis, we limited our analysis to the flash-ERG response, however, the retina is constantly bombarded by photons that generate noiselike electrical events. This asynchronous retinal activity (asynchronized ERGs or aERGs) can be recorded, and we recently presented preliminary results (Brassard-Simard et al., 2016) suggesting that physiologically meaningful information can be extracted from the aERG of normal subjects with the DWT. It would be interesting to extensively study the aERG in patients affected with different retinal disorders and/or at various stages using the DWT.

In the current research project, the DWT was able to detect delays in selected timefrequency components of the photopic ERGs (e.g. 20b, 40b, etc.) evoked from patients as well as in ERGs recorded from normal subjects in response to the brightest stimulus intensities. Indeed, the DWT can be used to detect significant peak time shifts, but given its lower temporal resolution, especially at low-frequency, detecting very subtle delays is more difficult. Of interest, in future studies, the stationary discrete wavelet transform (a slightly modified version of the DWT that has the same number of wavelet coefficients in each frequency band and hence a better temporal resolution) could theoretically decompose the ERG signal using the same DWT bands (20, 40, 80, 160 Hz) that were employed in this thesis, albeit with an enhanced temporal resolution. Using the stationary DWT (Mallat, 2009), we could add some temporal resolution to the DWT descriptors that were described herein. We could even study the luminance-dependence of their respective timing, or detect subtle peak time changes in one of the DWT components.

Of note, given that amplitude-independent descriptors of the DWT were very reproducible and very efficient for ERG segregations, I would recommend continuing the search for novel amplitude-independent parameters such as the 40-to-20b ratio, the %OPs, and the Hölder exponent. Meanwhile, I believe that one excellent way to start would be to always normalize the ERG waveform amplitude to the average of controls (e.g. and the magnification factor could be used as an additional descriptor) before DWT processing of the ERG. This would lead to positive and negative Z-Scores for each DWT descriptors (such as in Table 3), and the theoretically unique presentation of the Z-Score distribution (unique number patterns such as those in Table 3) for each pathological ERG could then be used to highlight significant differences between different ERGs or to create a dictionary of classes using advanced classifiers. Ultimately, a classifier could be used to make some sense out of normalized DWT data from several thousands of patients affected with different diseases (or stages), and detect disease-specific patterns (and stage-specific patterns) to improve clinical decisions and diagnoses.

Finally, as indicated above, the ERG remains the most objective diagnostic tool to confirm the diagnosis and monitor the disease progression of patients affected with a variety of severe retinal disorders. However, ophthalmologists continue to rely almost exclusively on funduscopy (imaging of the retina) to establish diagnostics, and as such, a complete retinal workup will always include fundus pictures, but rarely an ERG. This demonstrates that the ERG is still underused and limited in its diagnostic power. In contrast, in the last century, the diagnostic sensitivity and specificity of the electrocardiogram (ECG) have been improved to the point that, nowadays, it is almost inconceivable for a patient to go through a comprehensive cardiac workup that would not include an ECG. Of interest, in this thesis, the DWT method that we have presented has the promising potential to improve the diagnostic sensitivity and specify of the ERG to a level never reached before. We therefore hope that with additional experiments (such as the ones that we have proposed in the current section), the DWT will further improve our understand of the ERG signal and that with time, the origin of the various descriptors that we have identified will be precisely revealed. Clearly, the methods that we have developed will certainly improve the diagnostic utility of the ERG. Given the plethora of retinal diseases known to impair the normal functioning of the human retina, we are confident that our new descriptors of the retinal function will be an important addition to the tools that are currently available to the ophthalmologists.

7. Conclusion

In an attempt to improve the usefulness and the diagnostic power of the ERG I investigated the following question: "Can advanced analytical approaches (such as the FFT, CWT or DWT) extract additional useful diagnostic or physiological information from the photopic ERG"? To address this question, I isolated more than 10 novel highly reproducible ERG descriptors derived from the DWT and demonstrated that these descriptors were physiologically meaningful, diagnostically relevant and usable over a wide range of signal amplitudes and morphologies. Selected DWT descriptors can quantify distinct retinal events and can be independently affected by a given disease process, hence leading to the creation of novel diagnostic classes that better reflect the almost unlimited

ways by which the ERG can be altered as a result of normal and pathological conditions. Complementing ERG analysis with advanced DWT descriptors will allow more sensitive/specific quantifications of ERG responses, facilitate follow-up of disease progression, and identify diagnostically significant changes of ERG waveforms that are not resolved when the analysis is only limited to time domain measurements. In conclusion, advanced time-frequency approaches are clearly instrumental in extracting additional useful information from the ERG and could bring the analysis and interpretation of the ERG signal to the same state than that of the ECG and EEG. Our research thus opens the door to an exciting new era in ERG analysis, which will be beneficial to all involved, clinicians like patients.

CHAPTER VIII:

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CHAPTER IX:

APPENDIX

1. APPENDIX I: COMPUTATION OF THE FFT

Although the Fourier transform is defined as an infinite integral of sines and cosines, it is often rewritten using Euler's formula (for simplicity). Expressing sines and cosines as complex exponentials (with Euler) results in complex Fourier coefficients. The real part of the coefficients gives the amplitude (or prominence) of the frequencies present in the signal, and the associated phase is given by the imaginary part (Mallat, 2009; Van Drongelen, 2007). In this thesis, frequency domain analyses were limited to amplitude and/or power spectrums (i.e. the real part of coefficients). When the signal to be transformed is discrete, such as a time series, the applied Fourier is also necessarily discrete (Van Drongelen, 2007). Discrete Fourier transforms (DFT) are computed by numerical integrations and, with the development of powerful computers and algorithms, the computation of the DFT became very fast (Mallat, 2009). The most common DFT algorithm is the fast Fourier Transform, known as the FFT. Therefore, throughout this thesis, frequency domain analyses of the ERG were carried out using the FFT algorithm implemented as follows:

$$X(k) = \sum_{t=0}^{N-1} x(t) e^{-i(2\pi/N)tk}, \qquad k = 0, 1, \dots, N-1, \qquad (Eq. 1)$$

where X(k) represents the FFT coefficients, x(t) is the raw ERG time-series, and N the number of data points in x(t). Each FFT coefficient (i.e. X(k)) weighs the energetic contribution of a single frequency component to the signal so that an amplitude frequency spectrum can be illustrated by tracing the real part of X(k). Considering the standardized length (all ERGs were digitally resampled to have 512 data points) and associated resampled frequency (Fs = 3413.33 Hz) of ERG waveforms that were included in this thesis, we computed FFT coefficients for frequencies ranging between 0 and 1706.66 Hz (i.e. Fs/2) in increments of 6.66 Hz (i.e. frequency (1000 Hz) we limited our analysis to frequencies ranging between 0 and a maximum of 500 Hz to safely avoid artefactual contamination (such as that predicted by the Nyquist-Shannon sampling theorem (Nyquist, 2002).

2. APPENDIX II: COMPUTATION OF THE CWT

The continuous wavelet transform is a relatively recent alternative to Fourier analyses (FFT and STFT) that was first introduced in the eighties by Morlet and Grossmann (1984), following importance work in function theory by mathematicians such as Paul Levy, Raymond Littlewood, John Paley, Guido Weiss, Ronald R. Coifman, and David Marr. The continuous wavelet transform (CWT) of a signal is typically computed using the following equation:

$$CWT(a,b) = \frac{1}{\sqrt{a}} \int_{-\infty}^{\infty} x(t) \Psi^*\left(\frac{t-b}{a}\right) dt, \qquad (Eq.2)$$

where x(t) denotes the unprocessed time-series and $\Psi^*(t)$ denotes the complex conjugate of the mother wavelet (MW).

Of note, the MW must always observe some specific criteria to be considered as a valid wavelet. Firstly, a MW function $\Psi(t)$ belongs to the Hilbert space $L^2(\mathbb{R})$ and its integral must be equal to zero (null average). The latter condition involves that $\Psi(t)$ must oscillate between positive and negative values.

Furthermore, the MW is always characterized by a certain number of vanishing moments N. This property allows the MW to describe the fluctuations of the signal while being blind to undesired polynomial functions (of degrees lower or equal to N - 1). By selecting the number of vanishing moments, we can optimally adjust the trade-off between temporal accuracy and spectral resolution to better represent the chosen fluctuations in the signal. A high number of vanishing moments implies a more oscillatory wavelet with more spectral resolution, but lesser temporal accuracy and vice-versa (Strang & Nguyen, 1996).

By dilating/compressing the wavelet $\Psi(t)$ by a factor *a* and by translating it by a factor *b*, we obtain $\Psi((t - b)/a)$, producing a dictionary of time-scale atoms. Therefore, in Eq. 2, *a* and *b* represent the scale and translation parameters, while the term $1/\sqrt{a}$ is simply a normalization factor which allows the conservation of energy for any given scale *a*.

Finally, CWT(a, b) are the wavelet coefficients localized at scales *a* (frequency) and moments *b* (time) and represent the projection of the signal on the dictionary of timescale atoms. These wavelet coefficients can then be plotted in a time-frequency scalogram in which all the coefficients are positioned according to their coordinates *a* (frequency) and *b* (time). The latter provide a two-dimensional representation of the fluctuations of the signal localized simultaneously in time and frequency and where the magnitude of the coefficients reflect the prominence of fluctuations in the signal at any given time and frequency. To illustrate the time-frequency scalogram of the CWT, it is convenient to use the absolute value of CWT(a, b) and normalize it to the maximal value so that the time-frequency scalogram of the signal are shown as colored two-dimensional plots of CWT(a, b) in which minimum energy values are displayed in blue and maximal values in red.

Although there are theoretically countless types of MW that can be applied to the analysis of a given signal, in this PhD Thesis, we have solely considered the Morse family of wavelets (Lilly and Olhede, 2010, 2012), which is defined in the frequency domain as:

$$\psi(\omega) = \omega^n e^{-\omega^m} H(\omega) \tag{Eq.3}$$

where $H(\omega)$ represents the Heaviside step function and throughout this Thesis, n = 6 (vanishing moments) and m = 10 (order). The Morse wavelet was chosen for its good frequency resolution and since it has been successfully used in many recent studies (Worrell et al., 2012; Zerouali et al., 2013, 2014; O'Reilly et al., 2015).

3. APPENDIX III: COMPUTATION OF THE DWT

The discrete wavelet transform (DWT) of a signal is typically computed using the following equation:

$$DWT(j,k) = \int_{-\infty}^{\infty} x(t) 2^{-j/2} \overline{\Psi(2^{-j}t-k)} dt \qquad (Eq.4)$$

Where DWT(j,k) represents the wavelet coefficients localized at discrete scales j and discrete time k, x(t) designates the raw time series, and $\overline{\Psi}$ denotes the complex conjugate of the orthonormal mother wavelet.

One important characteristic of the DWT is the unique aspect of its time-frequency scalogram which is divided by a small number of levels (also termed level of decomposition). The number of levels n_j is determined by the number of samples (nS) in the signal according to:

$$n_i = \lfloor log_2(nS) \rfloor \tag{Eq.5}$$

Equation 5 reveals that for an optimal DWT decomposition, the number of samples in the signal should be a power of two (e.g. 256, 512, 1024, etc.). Furthermore, each level *j* is associated with a distinct bandwidth (*BW*) and a range of frequencies. The frequency interval $[f_{low}, f_{high}]$ of each level is given by:

$$[f_{low}, f_{high}] = [Fs/2^{j+1}, Fs/2^{j}]$$
(Eq. 6)

where *Fs* is the sampling frequency. As a result, the first level (j = 1; highest frequency components) includes frequency that are between *Fs*/4 and *Fs*/2. It is easy to show from the above Equation 6, that the *BW* of each level (inversely proportional to the spectral resolution) is equal to f_{low} .

Lastly, the number of wavelet coefficients nWC (directly proportional to the temporal resolution) computed at each level j is defined by:

$$nWC = nS/2^j \tag{Eq.7}$$

From the above Equations 6 and 7, we can conclude that when j is minimal, both the *BW* and the *nWC* are maximal. As a result, the temporal resolution is maximized and, the spectral resolution is minimized, in accord with the Heisenberg's uncertainty principle (Burrus et al., 1998). Since the Heisenberg's uncertainty principle governs each level, opposite time-frequency resolution properties are obtained when j is maximal (i.e. lowest temporal resolution and highest frequency resolution).

Of interest, given that there is a link between orthonormal wavelet bases and quadrature mirror filters (Mallat 1987; Daubechies, 1988), the DWT coefficients can be obtained using an extremely efficient algorithm, which was first described by Mallat (1989a). Since then, this algorithm has been termed the *fast wavelet transform* or *Mallat's algorithm* (Mallat 2009). The iterative process of Mallat's algorithm is elegantly simple. Briefly, a fast subband-coding algorithm successively decomposes the signal from its highest to lowest frequency components (e.g. level j = 1 to level $j = n_j$) using low-pass and high-pass half-band filters (HBF), which are essentially a set of two functions derived from the mother wavelet and respectively termed the scaling function and wavelet functions.

At the first DWT level, the raw signal x[n] is convoluted with the highpass HBF g[n] (i.e. wavelet function) and the lowpass HBF h[n] (i.e. scaling function) which is illustrated at Figure 5 and can be mathematically expressed as:

$$y_{high}[k] = \sum_{n} x[n] \cdot g[2k - n]$$
 (Eq. 8)

$$y_{low}[k] = \sum_{n} x[n] \cdot h[2k - n] \tag{Eq.9}$$

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Figure 5. DWT computation using the recursive fast wavelet transform algorithm of Mallat (1989).

The $y_{high}[k]$ output of g[n] is then subsampled by two and hence now has nS/2 samples (thus half the time resolution), but it only spans the frequencies Fs/4 to Fs/2 Hz (thus double the frequency resolution). These samples will constitute the first level of DWT coefficients (i.e. D_1 at Figure 5). According to the Nyquist's rule, the $y_{low}[k]$ output of h[n] is also subsampled by two, since the signal now has a highest frequency of Fs/4 Hz instead of Fs/2 Hz.

The $y_{low}[k]$ output (i.e. A_1 at Figure 5) is then passed through the same highpass and lowpass HBF for further decomposition. This recursive process continues until only two samples are left in $y_{high}[k]$. We thus obtain all levels of decomposition, each having half the number of coefficients of the preceding level. The DWT of the signal is then completed by concatenating all coefficients by starting from the last decomposition level. The DWT (or multiresolution vector) then have the same number of coefficients nWC as the number of samples nS in the original signal. As a result, the DWT is non-redundant. In order to reduce artefactual edge effects in our DWT studies (Torrence and Compo, 1998), each ERG was padded (before DWT computation) with 256 constant samples on both sides of the response by repeating the first and last value of the signal, respectively. With our parameters (i.e. 1024 samples per padded signal and a sampling frequency of 3413.33 Hz) and according to Eq.5, we thus obtain 10 levels of decomposition per DWT. The two last levels (9 and 10) were not considered in this PhD thesis and the padding was discarded after computation; obtaining eight-levels time-frequency plans of 150 ms in length (i.e. -20 to 130 ms), such as illustrated in Chapter III.

As detailed in Gauvin et al. (2015), each of the eight level has a distinct central frequency (level 1 = 1280Hz; level 2 = 640Hz; level 3 = 320Hz; level 4 = 160Hz; level 5 = 80Hz; level 6 = 40Hz; level 7 = 20Hz and level 8 = 10Hz). These levels (or frequency bands) do not quantify the contribution of the central frequency to the making of a given signal, but rather the contribution of a range of frequency/3). For example, level 7 of the DWT, whose central frequency is 20Hz, quantifies signal components that oscillate between 13.33Hz and 26.66Hz (i.e. $20Hz \pm 20/3Hz$). Consequently, some levels (such as the 20Hz and 40Hz) measured the contribution of the low-frequency components to the ERG (which could include the a- and b-waves) and other levels (such as the 80Hz and 160Hz) evaluated the contribution of the high-frequency components of the ERG (such as the OPs).

Additionally, another way to quantify the DWT scalograms consists in using the wavelet variance analysis (WVA). The latter characterises the variance of all wavelet coefficients obtained at each level (i.e., frequency band) of a DWT (Percival, 1995). WVAs are reported as a graph correlating the variance values against the corresponding DWT level (Gallegati, 2008; Park and Willinger, 2000). In this thesis, wavelet variances [expressed as the standard deviation (SD)] were derived using 256 wavelet coefficient values for the first DWT level down to 2 coefficients on the last level. The 8 SD values thus obtained were then plotted against their respective DWT level (Gauvin et al., 2015). From this representation, we defined the Δ -variance descriptor as the difference between

the SD obtained at level 6 and its estimation at the same level using the linear fit between levels 2 to 5 (see more details example in Gauvin et al., 2015).

The Hölder exponent is another descriptor that can be derived from the WVA of the DWT (Abry et al., 2002). This DWT descriptor is amplitude-independent and characterizes the irregularity of the waveform, a feature often associated with the complexity of a signal (Bishop et al., 2012; Sen et al., 2008). Of interest, the DWT offers an optimal scheme to estimate this exponent. This is because the variance of the wavelet coefficients *d* at a given level *j* varies as $Var(d_j) \sim 2^{jH}$. Accordingly, the Hölder exponent *H* can be estimated from the DWT variance-level plot (see example in Gauvin et al., 2015). Briefly, we compute the logarithm of the SD values at each level to obtain the log-variance diagram (see example in Gauvin et al., 2015). This process linearizes the curve. The estimation of the Hölder exponent is then simply reduced to the calculation of the slope over the alignment region in the log-variance plot. In this PhD thesis, the linear fitting was achieved between Levels 2 to 6 (as this region of the curve offered the best linear fitting).

Finally, the selection of a mother wavelet is mostly related to the selection of an optimal compromise between temporal accuracy and spectral resolution to represent the oscillations in the signal. This balance is controlled by the number of vanishing moments (VM) of the mother wavelet. A high number of VM implies a better spectral resolution, but lesser temporal accuracy and vice-versa (Strang and Nguyen, 1996). Various mother wavelets could have been used to extract the DWT descriptors described in this thesis. However, in this work, we wanted the local wavelet maxima descriptors of the ERG to analyse well-localized features of the signal (a-wave, b-wave, OPs, etc.). With zero vanishing moment, the Haar wavelet offers the best temporal accuracy, allowing the Haar wavelet coefficients to optimally identify the local features of a signal (Daubechies 1988; Daubechies, 1992). Moreover, the Haar wavelet has the most compact wavelet support, making it less sensitive to cross-contamination from adjacent fluctuations (such as that of the b-wave on the a-wave). Given the above characteristics, we selected the Haar wavelet to extract the LWM descriptors (20a, 20b, 40a, 40b, 80ops, 160ops) of the ERG throughout this thesis. In contrast, we wanted the WVA descriptors to analyse the complexity of the ERG more globally. The symmetric Daubechies wavelet (Lina and Mayrand, 1995) set
with two vanishing moments also has a compact support but given its morphology and added vanishing moments (better spectral resolution), it can better encompass the entire ERG waveform. The latter also makes this wavelet blind to low-order polynomials that are nonspecific to the ERG waveform and permit a sparse representation of the genuine ERG waveform. The latter allows a more robust calculation of the wavelet variance (SD) at each level of the DWT (Abry et al., 2002). Because of the above properties, we elected to use the SDW2 wavelet to quantify the WVA descriptors throughout this thesis.