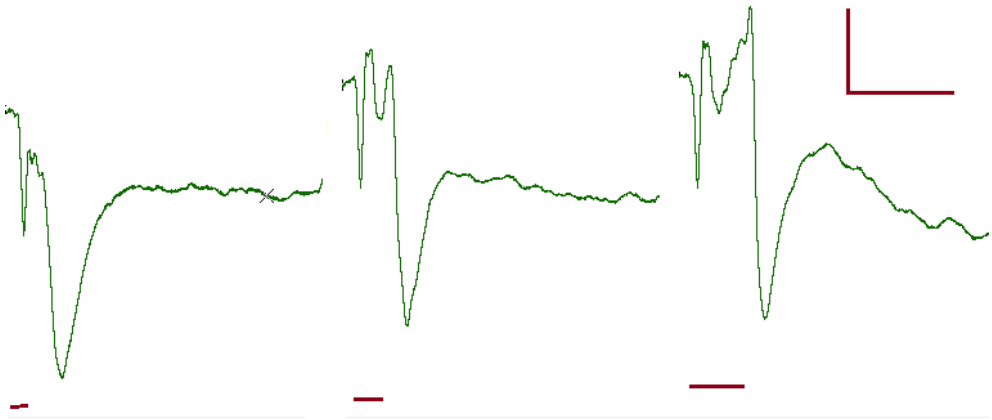
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

Evaluation of the retinal ON- and OFF-responses in the dog ERG



Charlotte Kristensson

Uppsala
2014

Degree Project 30 credits within the Veterinary Medicine Programme

ISSN 1652-8697
Examensarbete 2014:63

Evaluation of the retinal ON- and OFF-responses in the dog ERG

En utvärdering av det retinala ON- och OFF- svaret i hundens ERG

Charlotte Kristensson

***Supervisor**: Björn Ekesten, Department of Clinical Sciences*

***Examiner**: Helene Hamlin, Department of Clinical Sciences*

Degree Project in Veterinary Medicine

***Credits**: 30 hec*
***Level**: Second cycle, A2E*
***Course code**: EX0736*

***Place of publication**: Uppsala*
***Year of publication**: 2014*
***Number of part of series**: Examensarbete 2014:63*
***ISSN**: 1652-8697*
***Online publication**: http://stud.epsilon.slu.se*

***Key words**: Electroretinography, ERG, dog, cones, ON-response, OFF-response*
***Nyckelord**: Elektroretinografi, ERG, hund, tappar, ON-respons, OFF-respons*

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

SUMMARY

The aim of the study was to examine the retinal ON- and OFF-pathways of the canine electroretinogram (ERG). The ON- and OFF-pathways are used to distinguish objects from their backgrounds. A dark object is discernible from a lighter background using the OFF-pathway and the ON-pathway is used to descry a light object upon a darker background.

This study shows that the dog ERG has both ON- and OFF-responses and that these responses can be separated by prolonging the flash duration over 50 ms. The dog OFF-response waveform was more similar to the cat OFF-response than the human OFF-response (Frishman, 2006; Zrenner and Gouras, 1979).

In this study six female three-year-old Beagle dogs were included. Both background and flash stimuli were provided by LEDs (light emitting diodes) with a wavelength of 470 nm, a wavelength that is absorbed almost as effectively by the canine M/L-cones as by the S-cones. These stimuli gave ERGs with both ON- and OFF-responses. It was found that a background intensity of 40-60 cd/m^2 provided good ERGs with a-waves, distinct b-waves and a small positive OFF-response followed by an obvious negative OFF-response. When the relative flash intensities were varied from 12.28 to 12.8 log relative photons/m^2 per 100 ms- flash, all three curves were clearly readable. Flash intensities exceeding 12.8 log relative photons/m^2 reduced the negative OFF-response amplitudes.

Additional examinations ought to be made to gain knowledge about the OFF-responses of the S- and M/L-cones, respectively. This study also warrants further investigations on the cellular origin of the canine OFF-response, in particular the late negative OFF-component.

SAMMANFATTNING

I denna studie ingick sex treåriga beagletikar. Studiens syfte var att ytterligare undersöka hundars retinala ON- och OFF-banor med hjälp av elektroretinografi. De retinala ON- och OFF-vägarna används för att skilja ut ett objekt från bakgrunden. Via OFF-vägen blir ett mörkare objekt synligt när det kontrasteras mot en ljusare bakgrund och via ON-vägen kan ett ljust objekt bli synligt mot en mörkare bakgrund.

Studien visar att hundars elektroretinogram (ERG) har både ett ON- och ett OFF-svar, samt att dessa svar kan separeras när blixtdurationen överstiger 50 ms. Hundens OFF-svar visade mer likhet med det som ses hos katt än det OFF-svar som ses hos människor (Frishman, 2006; Zrenner and Gouras, 1979).

LED-lampor (Light emitting diodes) användes till både bakgrundsljus och blixtstimulering och dessa LEDar sände ut ljus med en medelvåglängd på ca 470 nm, en våglängd som absorberas nästan lika effektivt av hundens M/L-tappar, som av S-tapparna. Vi fann att en bakgrundsintensitet på 40-60 cd/m^2 gav tydliga ERGn med a-vågor, tydliga b-vågor och en liten positiv OFF-respons, som följdes av en större negativ OFF-respons. När den relativa blixtintensiteten varierades mellan 12,28 och 12,8 log fotoner/m^2 kunde b-vågorna, liksom OFF-responserna ses tydligt, men när intensiteten överskred denna nivå började amplituden hos den negativa OFF-komponenten minska.

Ytterligare studier behövs för att undersöka S- respektive M/L-tapparnas OFF-responser. Vår studie väcker dessutom frågan kring det cellulära ursprunget för hundens OFF- svar, särskilt den negativa OFF-komponenten.

CONTENTS

ABBREVATIONS USED

c= the speed of light in vacuum (299792458 m/s^2)
Cd= Candela
cGMP= cyclic guanosine monophosphate
CSNB= Congenital stationary night blindness
Different wavelength cones dog, (maximum absorption at; nm)
S = short (429)
M/L = median/long (555)
Different wavelength cones human (nm-nm):
S = short (400-500)
M = median (450-630)
L = long (500-700)
ERG= Electroretinogram
fERG= Flash electroretinogram
G-protein= guanosine nucleotide-binding protein
h= Planck's constant ($6{,}626076*10^{-34}$ Js)
iGluR = Ionotropic glutamate receptor
LED= Light emitting diode
mGluR= metabotropic glutamate receptor
ms= milliseconds
OP= oscillatory potential
Photopic= Light-adapted
PRA= Progressive Retinal Atrophy
RPE= Retinal pigment epithelium
Scotopic= Dark-adapted
W= Watt (=J/s= Joule per second)
λ_m= Maximum intensity/wavelength
µV= microvolt

INTRODUCTION

The retina is essentially our window to the world as it performs the first steps of the visual processing that eventually leads to conscious vision. Retinal function has been studied by scientists for generations. Since retinal anatomy is rather similar between all vertebrates, both human and veterinary ophthalmology may benefit from greater knowledge about retinal function in different species.

The eye perceives information about an object when the object is contrasted upon a different shaded background. This perception is relayed through the retina, by either of two separate routes termed the ON- and OFF- pathways. The OFF-pathway is operating when a dark object is visualized on a brighter background, while the ON-pathway is active when a light object is contrasted on a dark background. When a differently shaded object is introduced into the field of vision, for example when the predator sees the darker or lighter fur of the pray against the grass on the savanna, it is the ON- and OFF-pathways in the retina that convey the information to the brain.

Electroretinography is a non-invasive method of evaluating the function of the retina by measuring electrical potential changes caused by a light stimulus. Usually, brief flashes of light are used as stimuli, but the short interval between the onset and cessation of light in a flash (less than 5 ms according to the guidelines for clinical ERGs in dogs (Ekesten et al., 2013b) will produce an ERG where the ON- and OFF-responses are superimposed.

The purpose of this study was to further examine and describe the canine electroretinogram (ERG) in response to long duration stimuli under light-adapted conditions and thus separate cone ON- and OFF-responses. A protocol for separating ON- and OFF-responses would aid and enhance diagnosis of retinal diseases selectively affecting either the ON- or the OFF-pathway. An example of diseases selectively affecting the ON-pathway are forms of congenital night-blindness in the Briard breed of dog and in the Appaloosa horse, conditions that mimic Congential Stationary Night Blindness (CSNB) in human beings (Narfström et al., 1989; Nunnery et al., 2005; Sandmeyer et al., 2007; Miyake, 2006).

To the author's knowledge no studies exclusively examining the canine ON- and OFF- ERGs have been published as of this date. Hence, a study to gain fundamental knowledge, as well as proposing a protocol for clinical testing was needed.

LITTERATURE REVIEW

Retinal physiology and function

The retina is divided into the neuroretina and the retinal pigment epithelium (RPE). The RPE is a single layer of cells containing pigment granules and organelles for degrading waste products from the neuroretina. The RPE's function is to provide the neuroretina (mainly the photoreceptors) with nutrients, support and cleansing. Furthermore, a dietary vitamin-A derivate is transported from the bloodstream to the retina through the RPE (Kolb, 2006). The importance of this vitamin will be discussed below.

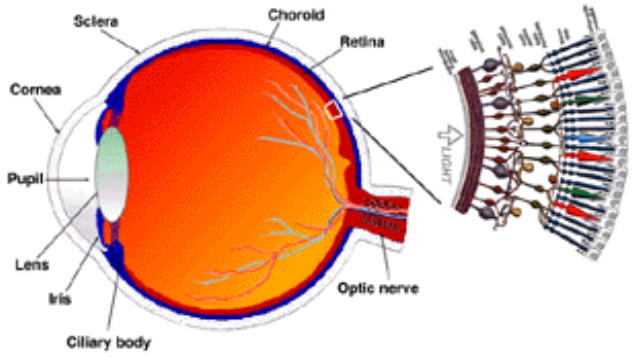


Figure 1a. Schematic of a human eye with an enlarged, cross sectional schematic of the retina.

Light must travel through the length of the eye and the neuroretina to reach the photoreceptors.

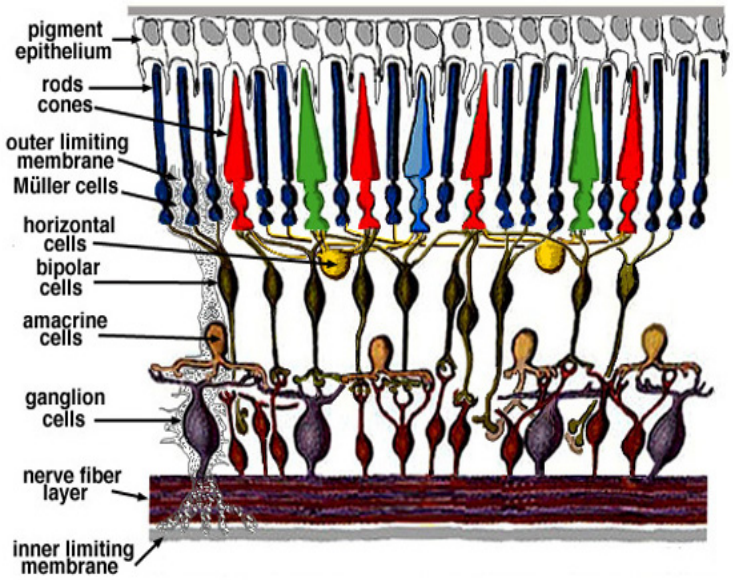


Figure 1b. Enlargement of the cross sectional schematic of the retina.

The cells and layers of the retina.

Figure 1a & b. Schematic of a human eye and retina. Courtesy of http://webvision.med.utah.edu/

The neuroretina consists of many cell types with different functions; photoreceptors (rods and cones), horizontal cells, bipolar cells, amacrine cells, ganglion cells and Müller cells (Figure 1b). All of these neuroretinal cell types come in a number of varieties and they all work together to convey the message initiated by capture of light by a photoreceptor. The inner layers of the neuroretina receive nutrition by and excrete waste products through the retinal blood vessels.

Retinal processing of light

Light enters the eye through the pupil and reaches the neuroretina at the inner limiting membrane; however it is the photoreceptors adjacent to the RPE that catches the light (figure 1).

First order neurons; the photoreceptors

A photopigment is a dual molecule consisting of a pigment (opsin) that determines the light wavelengths being absorbed and a light-sensitive chromophore (a vitamin-A derivative) (for review see Ofri, 2013). In the normal human retina, there are four different photoreceptors present; rods, S- (short-wavelength sensitive), M- (medium-wavelength sensitive), and L-cones (long-wavelength sensitive), whereas dogs have only three; rods, S-, and M/L- cones. Rods are highly light sensitive and functions only in dim light. As the light increases, they become saturated. Cones however, are designed to function in bright light. In humans, conditions affecting the function of one or more of cone classes exist, of which the most common types are green-red color blindness (Neitz and Neitz, 2000). In such a condition where only two classes contribute to cone vision (called dichromacy), most often either the M- or L- cone are lacking or nonfunctional and this condition renders the affected individuals with a lesser or missing capability of distinguishing green or reddish colors.

After the initial absorption by the chromophore, the light acts as particles, photons (for review see Ofri, 2013). The energy of a photon affects the chromophore so that its conformation is changed; this structural change leads to separation of the photopigment and the chromophore. The freed photopigment/opsin diffuses through the membrane and activates other molecules. One of the activated molecules is a specific G-protein (transducin) and the final result of the chain of reactions, the phototransduction cascade, induced by this protein is a reduction in intracellular cGMP. The reduced cGMP levels act on the intracellular Ca^{2+} concentration by a negative feedback system, which further decreases the cGMP. A reduced intracellular level of cGMP will inactivate the transmembrane Na^{+}/Ca^{2+}-K^{+} pumps (CNG channels). This channel closure stops the influx of positive ions (Na^{+}/Ca^{2+}), leaving the cell hyperpolarized (electronegative). In the dark, the photoreceptors are instead kept depolarized (electropositive) because of the constant Na^{+}/Ca^{2+} -influx (the dark current). The depolarized cell constantly releases glutamate and when the photoreceptor is activated/ hyperpolarized this glutamate release is terminated. The glutamate molecules affect the cells synapsing with the photoreceptor by initializing further cell membrane polarization. The glutamate molecule can be described as a biochemical messenger, a transmitter, regulated by the energy in photons.

Second order neurons; bipolar cells

The photoreceptors transform light from pure photon energy into biochemical messengers, which conveys the signal to other types of cells in the retina. Laterally, the photoreceptors synapse with horizontal cells and vertically they synapse with bipolar cells (For review see Kolb, 2006). Bipolar cells then further transduce signals from the photoreceptors to ganglion and amacrine cells. Cones synapse to cone-bipolar cells. In humans, these synapses are made either to single foveal/midget bipolar cells or as 5(-20) cones to 1 diffuse cone bipolar cells (for review see Kolb, 2006; Ofri, 2013). The glutamate flow in these synaptic clefts is

regulating the polarization of the bipolar cells that are subdivided into either ON- or OFF-bipolars. The ON-bipolar cells express metabotropic glutamate receptors, mGluRs, which are slow, inhibitory receptors depolarizing the cell. OFF-bipolar cells express ionotropic glutamate receptors, iGluRs, which in turn are fast, excitatory receptors that hyperpolarize the cell. These ON- and OFF- bipolar cells are the start of the ON- and OFF-pathways.

The ON- and OFF-pathways

The ON- and OFF-pathways provide information about the brightness contrast of the image received from the photoreceptors that can be processed even further in other, specialized types of cells (third order neurons; amacrine- and ON-/OFF-ganglion cells). The OFF-pathway is in effect when a dark object is pictured upon a lighter background (this text for instance) and the ON-pathway is used to detect the opposite. These pathways work independently and if one malfunctions, the other may still be intact. Genetic diseases affecting the ON-pathway specifically are known in humans, dogs and horses (for review see Miyake, 2006; Narfstrom, 1989; Nunnery et al., 2005; Sandmeyer et al., 2007). These ailments render the affected individuals with complete or partial nightblindness. Daytime vision is however less affected.

Regulation and support

Horizontal cells connect with rods and cones and regulate light adaptation in part (for review see Kolb, 2006; Ofri, 2013). Some horizontal cells are also important for color processing. The horizontal cells connect only to cones and not to rods. They also connect with bipolar cells and attenuate their signals. The amacrine cells are predominantly situated in the inner nuclear layer and function much like the horizontal cells here, as they modulate the signals from the bipolar cells on their way to the ganglion cells. Müller cells are the supportive cells of the retina; they help maintaining structure and nutrition, as well as with waste management.

Electroretinography

The retina can be visualized using an ophthalmoscope. However, its actual functional integrity can only be evaluated with the aid of specific diagnostic tools measuring retinal response to a light stimulus. An electroretinogram (ERG) measures the electrical currents that develop when light reaches the retina and the cells change polarity.

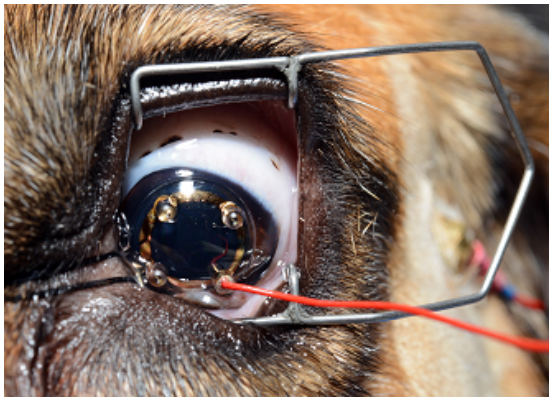


Figure 3. Preparation for an ERG. A contact lens electrode is placed directly on the cornea and a lid speculum is retracting the eyelids.

Procedures

The flash ERG, fERG, is registered by placing electrodes in contact with the cornea and caudal to the temporal canthus (figure 3). A ground electrode is also placed in an indifferent position, such as on top of the skull (figure 4). The electrodes will register the currents that develop as the retinal cells are polarized by light stimuli. These polarization currents are very small and amplifiers are used to magnify the signals. The signals are displayed by specialized software, which additionally saves the data. Furthermore, responses to a series of flashes are often averaged to minimize artifacts and electrical noise. To maximize light input (both quantity and distribution) to the retina and thereby maximizing the responses, the pupil is dilated with a mydriatic agent and the eyelids are kept open with a speculum.

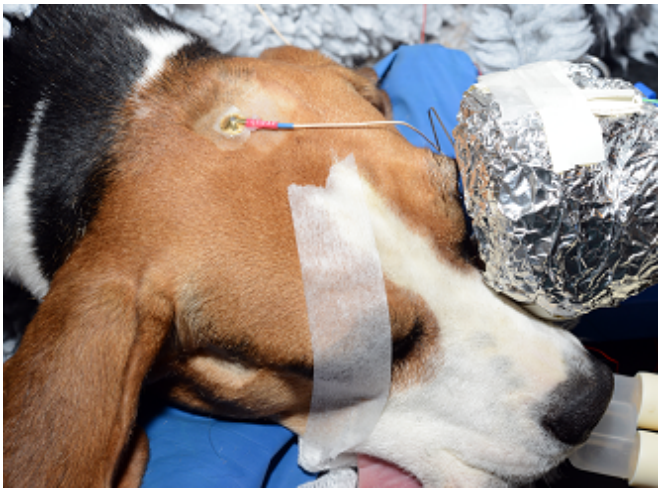


Figure 4. Preparation for an ERG. The dog is placed in ventral recumbency and equipped with a ground electrode. The opposite eye is kept closed.

In an ERG, the electrical potential will be shown on the ordinate (amplitude in µV), and plotted against the time on the abscissa (in ms). A typical light-adapted dog ERG is shown in figure 5.

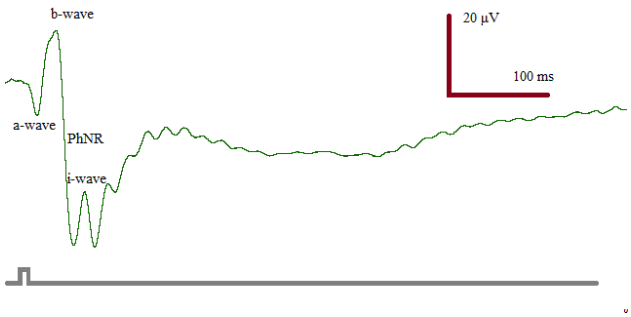


Figure 5. A light adapted dog ERG. For explanation see under "Interpretation of the ERG". Background intensity of 30 cd/ m^2 and flash intensity of 11,62 log relative photons/m^2. The bottom grey line represents flash duration (5ms).

Many factors influence the shape of an ERG. Shape of the head, pupil size and position of the eyeball etc. are differences, which need to be considered when performing and evaluating an ERG. Also physiological differences, such as number of and type of retinal cells stimulated, oxygenation and blood glucose levels affect the ERG. It is recommended that comparison of an ERG is firstly made within the same or similar breeds rather than between all sorts of dogs. It is important that the evaluated animal stays very still, because movements and muscle spasms will affect the ERG. This is usually

accomplished by keeping the patient under general anesthesia. Medications given will however also affect the ERG, because they may affect the chemical messengers between the cells. Because of this it is important to use the same anesthetics when obtaining reference values, as when evaluating a patient since the effects will then be similar. The effect of hypoxia and hypercapnia are decreased ERG amplitudes. Therefore, it is important to maintain sufficient ventilation during the procedures (Linsenmeier et al. 1983). This is readily accomplished by intubating the dog and providing oxygen.

Light stimulation

Different light stimuli can be used to examine different cell types and their functionality. The light must be evenly dispersed over the entire retina (Gouras, 1970) and this is most often facilitated using a Ganzfeld (full-field dome; Figure 6).

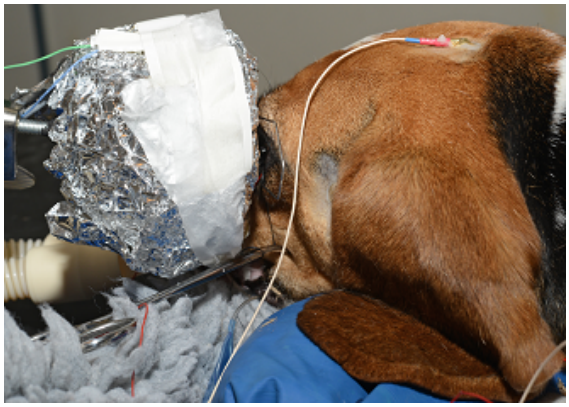


Figure 6- Ganzfeld dome.

Left: Customized full-field dome for ERG recordings in dogs.

Right: A customized mini-ganzfeld positioned to provide evenly dispersed light onto the left retina of a Beagle.

By changing the background light level, rods and cones can be separated and the ERG will change accordingly (Gouras, 1970). The degree of background illumination that provides a good cone ERG has been examined in human beings (Peachey et al., 1989; Peachey et al., 1992; Wali & Legiure, 1992; Rufiange, 2003). Wali and Leguire (1992) used higher intensity background stimulation, and found a previously not described phenomenon they called the photopic hill. This concept describes how the b-wave (see next section) amplitude of a light-adapted ERG gradually increases with increasing background light stimuli up to a certain point, and thereafter starts to decline with further increase in background luminance.

By changing the spectral composition of the light source, for instance by using coloured LEDs, different classes of cones can be evaluated. The responses of interest can be enhanced if a chromatic background is used to suppress the responsens from the other cone classes and rods (selective chromatic adaptation; see for instance Zrenner and Gouras, 1979). The flash stimuli can also be varied both in intensity and duration to evaluate different cell types.

Updated guidelines for canine ERG registration have recently been published and provide a description of the basic techniques for obtaining rod, mixed rod/cone and cone responses in the canine ERG (Ekesten et al., 2013b).

Interpretation of the ERG

Light reaches the retina and subsequently polarizes the neuroretinal cells as previously described. When a noninvasive, corneal ERG is recorded, a hyperpolarization will normally be shown as a negative deflection from baseline and depolarizations will appear as positive wavelets.

A simplistic explanation of the cone ERG is that the first negative deflection, the a-wave, essentially represents hyperpolarization of the cones. The second, positive deflection, the b-wave, mainly represents the bipolar cells, both ON- and OFF-bipolars. When a long duration flash is used, the b-wave is driven by the depolarizing, ON-bipolars and an additional positive deflection, the d-wave, is present. This wave represents the OFF-bipolar response. Because the OFF-bipolar cells are inhibited during the flash, it is logical that when they are activated (light is turned off) they become depolarized, hence producing a positive wavelet after the cessation of the flash.

The ERG is however a bit more complex than this and a few more features can be found and used when evaluating retinal function. Figure 7 shows an ERG elicited by a short duration flash (5 ms).

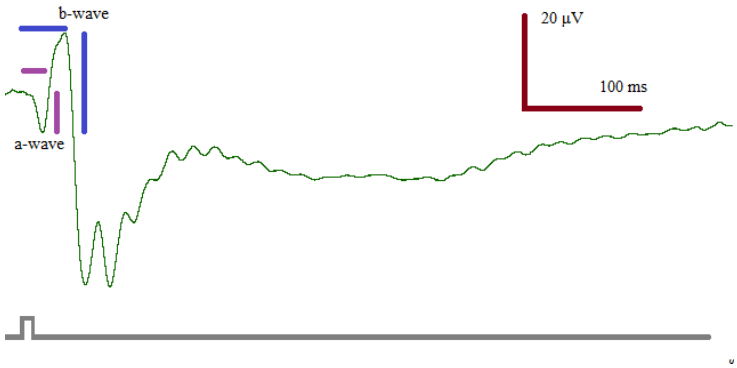


Figure 7. A typical photopic ERG response elicited by a short duration flash. The a-wave is the first negative deflection. The b-wave is the first positive wavelet. The implicit times and amplitudes of the curves are measured (ms and µV) as shown by the horizontal and vertical purple (a-wave) and blue (b-wave) lines respectively.

The a-wave

The light-adapted a-wave (first negative wave) is the joint result of cone photoreceptors and OFF-bipolar cells (Bush and Sieving, 1994). The photoreceptors dominate the initial part of the a-wave, whereas the OFF-bipolar cells and the ON-bipolar cells merely attenuate the later part of the wave. The ERG will only show one curve where the contributions are superimposed.

The b-wave/ ON- response

In 1994, Sieving et. al. investigated the origin of the b-wave and concluded that it originates from depolarizing (ON-) and hyperpolarizing (OFF-) bipolar cells. By injecting synaptic blockers he could conclude that it is the depolarization of ON-bipolar cells that initiates the b-wave, but that the amplitude and shape of the curve is regulated by the opposing hyperpolarization of OFF-bipolar cells. The summed deflection is shown in the fERG.

Oscillatory potential

The term oscillatory potentials, or OP's, is used for the fluctuations on the ascending limb of the b-wave curve, which can be seen in many species (see Ekesten, 2013a for review). Cats and dogs usually present with three to five of these oscillations on the b-wave. The origin of each of these wavelets is still debated, however they are thought to have a strong inner retinal component.

The i-wave

An i-wave is also sometimes present in a light-adapted animal ERG (Ekesten, 2013a). It is a positive deflection superimposed at the end of the descending limb of the b-wave and it is currently considered to originate from the optic nerve and/or from the retinal ganglion layer.

The d-wave/ OFF-response

In the primate light-adapted ERG, when a flash stimulus with a duration exceeding 50 ms is used, yet another wave, the d-wave (figure 8), is present (Sustar et al., 2006). This wave is not apparent when short flashes are used because it is then superimposed on the b-wave (Sieving, 1993).

The d-wave is a positive wave and it appears after cessation of the flash. The d-wave is produced when hyperpolarizing cone bipolar cells are transiently being depolarized almost simultaneously as the photoreceptor hyperpolarization is terminated in primates (Frishman, 2006). These simultaneous actions are inseparable in the flash electroretinogram and only a short positive deflection is apparent. The d-wave is often referred to as the OFF-response. The d-wave is believed to be larger in species having many cones (such as the ground squirrel with an all-cone retina) (Frishman, 2006).

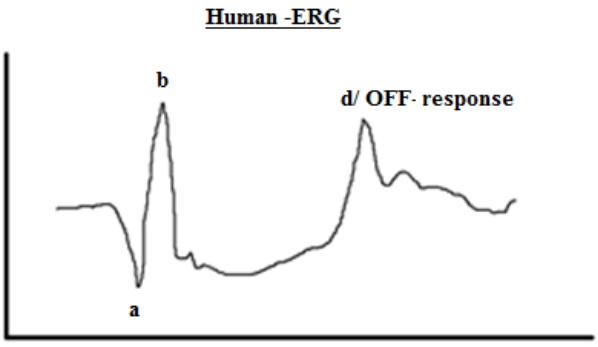


Figure 8a. A schematic of a light adapted human ERG with a positive d-wave after cessation of flash

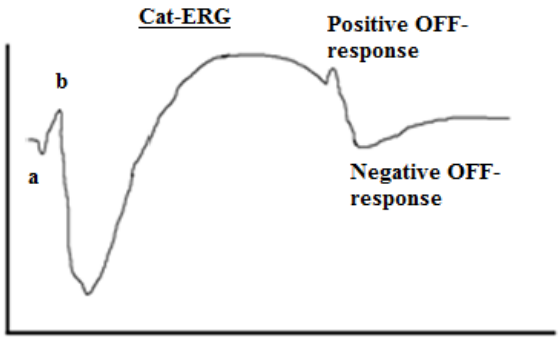


Figure 8b. A schematic replication of a light adapted cat ERG from Zrenner and Gouras (1979) with a positive d-wave after cessation of flash followed by a negative OFF-response.

Zrenner and Gouras (1979) found that an OFF-response is present in the cat ERG. This cat OFF-response was slightly different from the primate OFF-response (Figure 8). The authors saw that the cat OFF-response showed a small positive d-wave representing the *positive OFF-response* (d-wave) but also a negative deviation after the d-wave, which represents the *negative OFF-response* (figure 8b). In their study, Zrenner and Gouras also found that the cat d-wave most likely originated from the M/L cones since only the negative OFF-response was present when the S-cones were stimulated, whereas the M/L-cones had both a *positive-* and a *negative OFF-response*. The primate OFF-response does not show the same pronounced post d-wave negativity as found in the cat (Frishman, 2006; Zrenner and Gouras, 1979) (figure 8a). The d-wave has not been examined in the dog (Ekesten, 2013a).

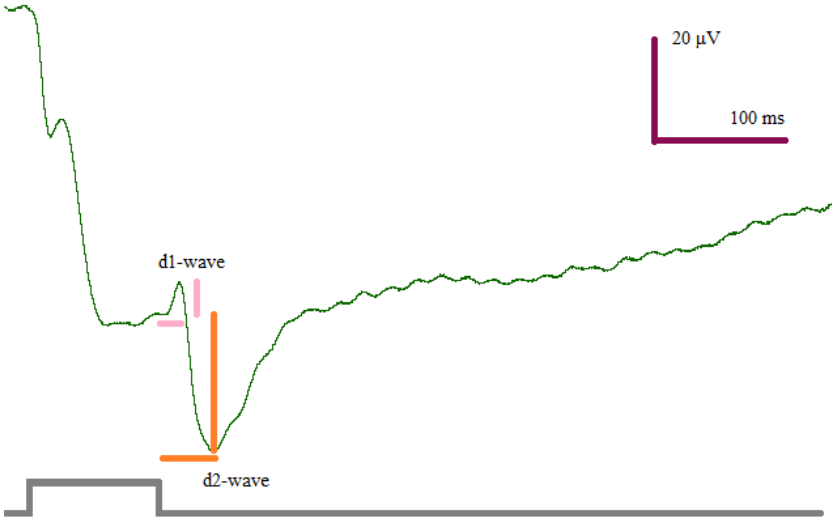


Figure 9. ERG to a long duration flash (100ms). The d-wave is present. Implicit time and amplitude are measured as shown in figure 7. The pink lines show how the d1-wave is measured and the orange the same for the d2-wave. The black tracing at the bottom indicates the stimulus.

The photopic negative response

The photopic negative response (PhNR) is another component of the cat-, human- and monkey-ERG (Frishman, 2006). The PhNR is a late negative response present in a light-adapted ERG seen when the retina is stimulated with a bright flash. It is first present after the b-wave but also appears later, after the d-wave. The origin of this wave is not yet fully understood but it is considered to arise in the retinal ganglion cells and their axons. It is measured from baseline to trough after b-wave.

Historical interpretation

The ERG-interpretation used today, is however not the first description of the retinal polarization. Another, earlier, description of the electrical potential changes registered in an ERG was made by Ragnar Granit (1933). He examined retinal potentials in cats under different degrees of general anesthesia and detected three components of the ERG,

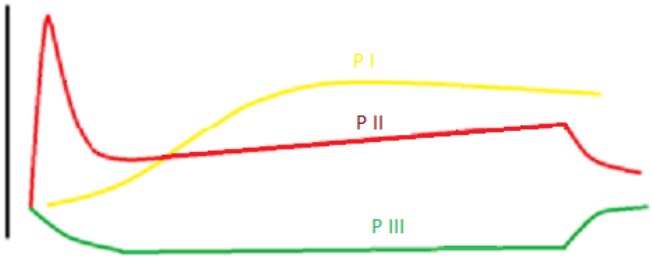


Figure 10. Schematic Replication of Granits (1933) ERG curve.

which he named process I, II and III (P-I, P-II and P-III) in order of their disappearance. Figure 10 is a schematic drawing of the waves he registered. The a-wave is the first part of P-III, the b-wave is the summation of P-II and III (Ekesten, 2013a). The P-I, or c-wave, is a large positive wave originating in the RPE (cellular origin first described by Noell in 1952).

The description of Granit and his acronyms are at times still used to describe the components of the ERG.

Clinical applications for the ERG in ON- and OFF- pathway dysfunctions

Since there are no existing protocols for evaluating ON- and OFF-pathways in dogs the clinical applications are yet unknown. Future applications are therefore only speculative, but may include earlier and accurate diagnosis of forms of stationary night blindness in both dogs and horses and possibly also other yet unknown diseases of the ON- and OFF-pathways.

MATERIALS AND METHOD

Dogs

Six female Beagle research dogs, age 3.2 years (SD: ±26 days), weight 13±1.3 kg from the Department of Clinical Sciences at the Swedish University of Agricultural Sciences (SLU) were included in the study. The use of animals in this study was approved by the Uppsala regional Ethical Review Board and the experiments were conducted in accordance with the guidelines of the Association for Research in Vision and Ophthalmology (ARVO).

Prior to the ERGs, a clinical examination including body condition score, posture and gait, hydration status, auscultation of heart and lungs, palpation of lymph nodes and abdomen was performed. An ophthalmic examination including testing of palpebral reflexes, direct and consensual pupillary light reflexes, dazzle reflex, menace response, a simple test of vision using falling cotton balls, slit lamp biomicroscopy (Kowa SL-15, Kowa Optimed Deutschland GmbHand, Düssseldorf, Germany), direct (Heine Beta200 ophthalmoscope, HEINE Optotechnik, Herrsching, Germany) and indirect ophthalmoscopy (Topcon ID-10, Topcon Corporation, Tokyo, Japan), and tonometry (Tono-Pen XL, Medtronic Solan, Jacksonville, USA) were also performed. Results of the examinations are shown in table 1. There was no history of previous ocular or systemic disease in any of the dogs according to their keeper.

Table 1. Findings at clinical and opthalmologic examinations in the six Beagle dogs included in the study. n.a.d.= no abnormal discovery

	DOG					
Examination	1	2	3	4	5	6
Clinical	n.a.d.	n.a.d.	mild tonsillitis	n.a.d.	n.a.d.	n.a.d.
Ophthalmic	n.a.d.	n.a.d.	mild disthichiasis	n.a.d.	n.a.d.	n.a.d.

Preparation

The dogs were sedated with 0.04-0.05 mg/kg acepromazin i.m. (Plegicil vet 10 mg/ml, Pharmaxim, Helsingborg, Sweden) depending on their temperament. If the dog was calm the lower dose was used, whereas more anxious dogs received the higher dose. General anesthesia was induced with 6-8 mg/kg propofol (Lipuro 10 mg/ml, B. Braun Melsungen AG Melsungen, Tyskland) i.v. All dogs were intubated and maintained on isoflurane (Attane vet., 1000mg/g, Piramal Health Care UK Limited, Northumberland, Great Britain) and oxygen. Hydration was maintained using intravenous infusion (Ringer-Acetate, Baxter Healthcare Corporation, Chichago, Illinois, USA) at a dose calculated from a daily dose of 40 ml/kg given at a rate of approximately 60 ml/h.

Pupils were dilated with 2 drops each of the topical mydriatic agents cyklopentolate (Cyclogyl 1%, Alcon Laboratories, Inc. Fort Worth, Texas, USA) followed by tropicamide (Mydriacyl 0.5%, Alcon Laboratories, Inc. Fort Worth, Texas, USA), each instilled twice. The two agents were given 5 minutes apart with a 15-minute interval between the first and second instillation. The last drop was given approximately 30 minutes prior to the experiment. Pupil dilation was evaluated before and after the experiments.

Artificial tears were used as coupling agent between the ERG electrode and the cornea and also after the experiment to keep the cornea moist (Comfort Shields, 0.15%, Vétoquinol Scandinavia, Åstorp, Sweden).

The dogs were placed in ventral recumbency during the ERG. The third eyelid was pulled aside by a stay suture (silk 4-0) and the eyelids were kept open using a lid speculum. Only one eye was used during the experiments and the opposite eye was kept closed using standard surgical tape. Dogs were monitored with a pulse oximeter (TuffSat, GE Healthcare, Louisville, CO, USA) and an esophageal phonendoscope during the entire anesthesia.

Background and stimulus lights

A customized mini-ganzfeld (figure 11) with built-in LEDs (YSF-B319EY; Yoldal CO., Ltd. Zhonghe, Taipei) was used for stimulation, as well as background light. Both the light stimulus and background light was driven by a signal generator (Siglent; SDG-5082, Ferner elektronik AB, Järfälla, Sweden). The stimuli, as well as the registration of signals, were synchronized by a general purpose electrophysiology system (PowerLab/8SP and Scope 4 software, AD instruments Ltd, Castle Hill, Australia).

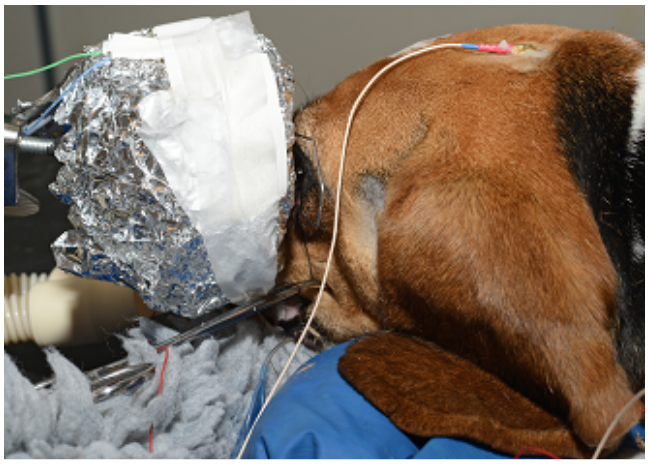


Figure11. Light stimulation. The customized mini-ganzfeld is placed in front of the left eye.

The light intensities were measured for both background light and flash by a

research grade photometer (IL 1700, International Light Ltd, Newburyport, MA, USA) before the experiments were started. Light intensities were measured in both cd/m^2 and relative W/m^2. The photometer was used without a color filter, but not calibrated in W/m^2 for use without the filter. Hence, the readings were in an arbitrary unit based on W/m^2, which allowed calculation of the relative number of photons/m^2/s (figure 12). These relative intensity values where then used when different stimuli were compared.

ERG recordings

The ERGs were recorded using a corneal contact lens electrode (JET-lens electrode, Universo, Switzerland). A skin reference electrode (Gold Disc Electrodes, F-E5GH, Natus Neurology Incorporated, Grass Products, Warwick, USA) was attached to the skin approximately three centimeters caudal to the temporal canthus using conductive paste (Ten20 Conductive, D.O Weaver & Co, Aurora, USA) and a similar ground electrode was placed on the vertex. The impedance between the electrodes was measured with an impedance meter (Model F-EZM5, GRASS Astro-Med inc., West Warwick RI, USA). The resistance was never allowed to exceed 5 kΩ.

The signals were amplified through a preamplifier (Iso-Dam, World Precision Instruments Inc., Sarasota, FL, USA) and a combined amplifier and AD-converter (PowerLab/8SP AD instruments Ltd, Castle Hill, Australia). Special softwares for registration of electrophysiological data and post-experiment analysis were used (Scope 4 and LabChart 7 reader, ADInstruments Ltd, Castle Hill, Australia).

E=H*C/λ= 4,22648428*10^-19 J (Ws)

n= P/E P= measured W/m2

Figure 12 a. Calculation of log relative number of photons/m^2. The number of photons (n) is obtained by inserting an approximation of the calculated energy (E) emitted by the LEDs used in this experiment. The energy of a photon is given by the formula: E= hc/λ. The energy (in relative W/m^2) measured with the photometer, P, is then used to calculate the relative number of photons, n; n= P/E

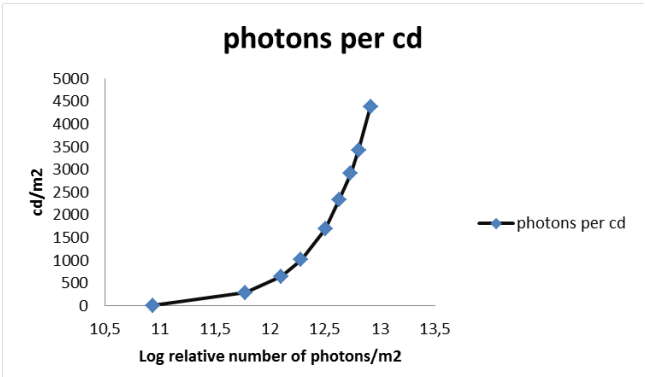


Figure 12b. Relative number of photons/m^2/s (n) per cd/m^2 intensity

Protocol

Intensity-response series were conducted at different background intensities and the appearance of the b- and d-waves were examined. Table 2 shows the different flash intensities and durations that were used on each background.

Table 2. Protocols used in the study. Table 2a shows the flash intensities and durations used on the 20cd /m^2 background. An "X" shows that a certain flash intensity (per area unit and second) of a certain duration was used in one dog. Tables 2b trough 2e show the flashes used on the 30, 40, 60 and 83 cd/m^2 backgrounds, respectively.

2 a.

20 cd/m2 background intensity

Burst	Time (ms)				
log relative photons/m2/s	5	25	50	100	200
10,94	x			x	
11,78	x			x	
12,1	x			x	
12,28	xxx			xxxx	
12,5	x			x	
12,63	xxx	xx	xx	xxxx	
12,81	x			x x	
12,91	x			x	

2 b.

30 cd/m2 background intensity

Burst	Time (ms)				
log relative photons/m2/s	5	25	50	100	200
10,94	x			x	
11,78	x			x	
12,1				x	
12,28				x	
12,5				x	
12,63	x	x	x	x	x
12,81				x	
12,91				x	

2 c.

40 cd/m2 background intensity

Burst	Time (ms)				
log relative photons/m2/s	5	25	50	100	200
10,94	x			x	
11,78	x			xxx	
12,1	x			x	
12,28	x			xxx	
12,5			x	xx	
12,63	xx	xx	xxx	xxxx	xx
12,81			x	xxx	
12,91			x	xxx	

2 d.

60 cd/m2 background intensity

Burst	Time (ms)				
log relative photons/m2/s	5	25	50	100	200
10,94	x			x	
11,78	x			x	
12,1	x			x	
12,28	x			x	
12,5	x			x	
12,63	x	x	x	xx	
12,81	x			x	
12,91	x			x	

2 e.

83 cd/m2 background intensity

Burst	Time (ms)				
log relative photons/m2/s	5	25	50	100	200
12,28				x	
12,63				x	
12,81				x	
12,91				x	

Dogs were light-adapted for ten minutes at each background intensity. A two-minute interval was used between different flash colors (Erica Rydhed's degree project) and also in-between flash intensities. Averaged responses from 20 - 30 flashes were obtained for each flash duration and intensity.

Dog 5 was excluded due to pupil constriction at the end of the experiment. All the other dogs had dilated pupils both before and after the trials.

Measurements

In the ERG, oscillatory potentials (OPs) of various amplitudes are superimposed on the b- and d-waves. Therefore, we filtered out the OPs as shown in figure 13 (green curve) and all measurements were later made from the filtered curves.

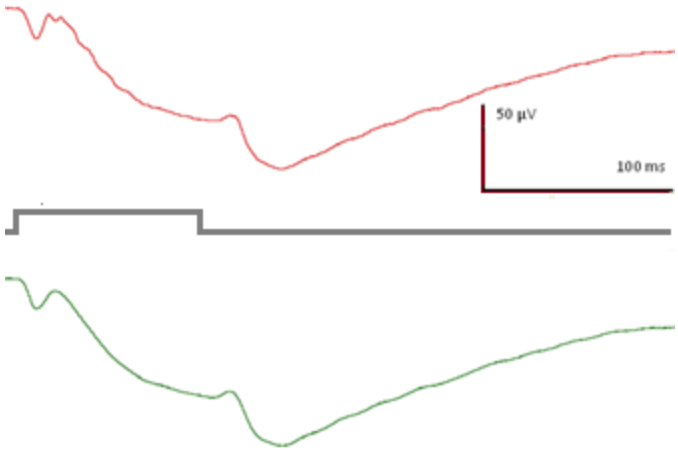


Figure 13. The upper curve is the original curve and the lower curve is the filtered curve.

The middle curve shows the flash duration.

The implicit time for the b-wave was measured from start of flash (M) to the peak of the b-wave (figure 14). The implicit times for the d1- and d2-waves were measured from cessation of flash (m) to peak of d1 and trough of d2, respectively. The b-wave amplitude was measured from the

trough of the a-wave to the peak of the b-wave and the d1- and d2-wave amplitudes were measured from level of m (amplitude at cessation of light) to the peak and trough, respectively.

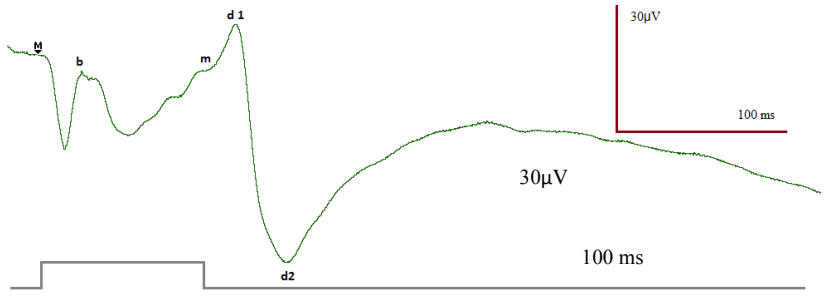


Figure 14. The ERG of a dog from a flash of 11.63 log relative photons/m^2 upon a 40 cd/m^2 background.
M is the start of flash and m its cessation. b=b-wave, d1=d1- wave and d2=d2-wave.

STATISTICAL ANALYSIS

Means and median values were obtained using Microsoft Excel. JMP was used to fit linear models using standard least squares, as well as testing if residuals significantly deviated from a normal distribution using the Shapiro-Wilk statistic (JMP version 10.0.2, SAS Institute Inc., Cary, North Carolina, USA). P-values less than 0.05 were considered significant.

RESULTS

When short duration stimuli were used a b-wave was present, but a separate d-wave could not be observed. However when flash duration was increased to 50 ms or longer, the d1- and d2-waves became discernible.

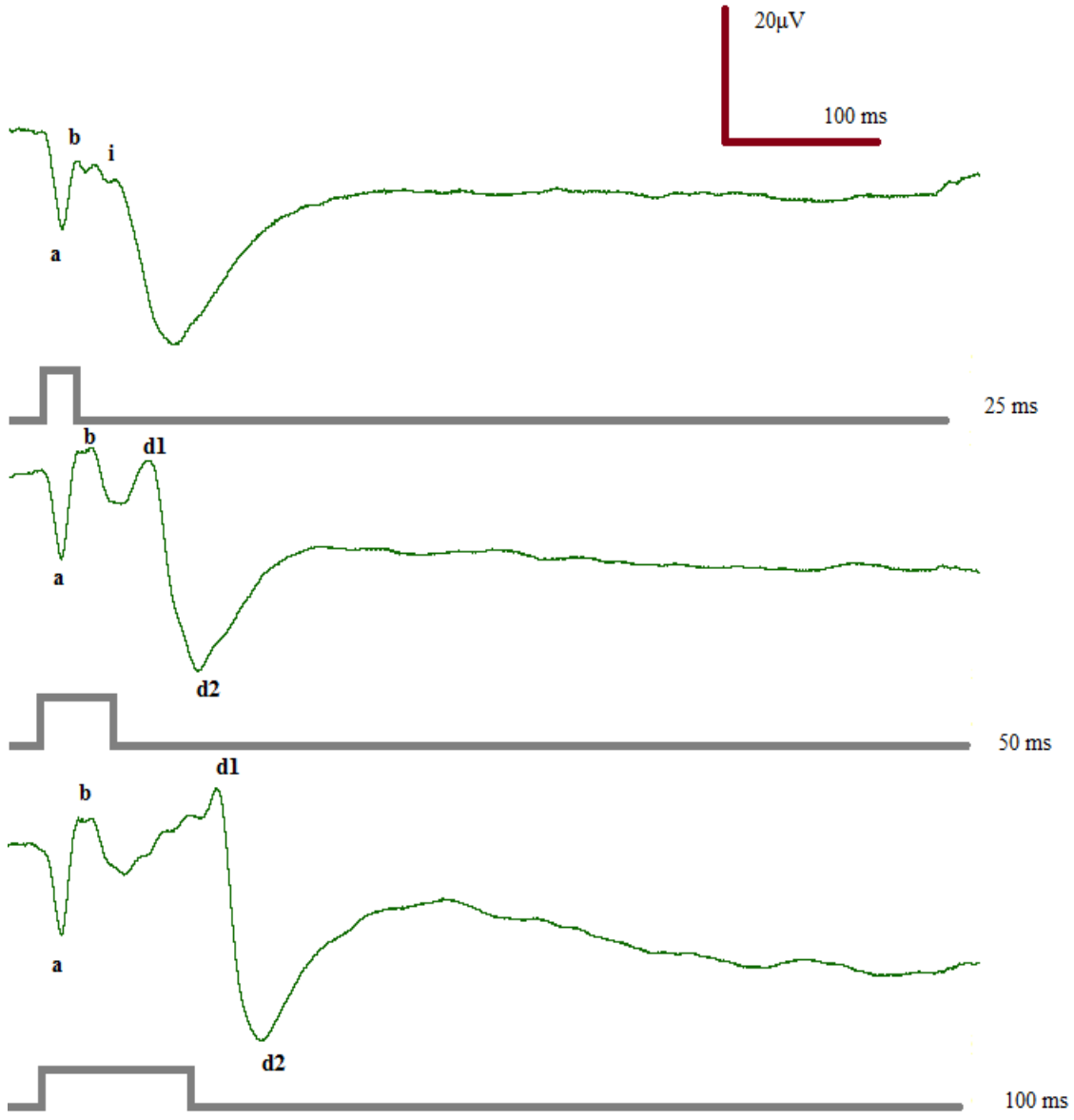


Figure 15.Effect of prolonging the flash duration. Curves at a 60 cd/ m^2 background and a flash intensity of 12.63 log relative photons/ m^2/s.

Upper ERG, 25 ms stimulus; only a- and b-waves are discernible.
Middle ERG, 50 ms stimulus; distinct d1- and d2- waves are seen.
Lower ERG,100 ms stimulus: the d-waves are present and move away from the b-wave when the flash duration is further increased.

Evaluation of implicit time

Figure 16 shows the minimum, median and maximum implicit times, for b-, d1- and d2-waves for all dogs from all background and flash intensities used in the study. The d2-wave implicit time varied more than the b- and d1-waves did.

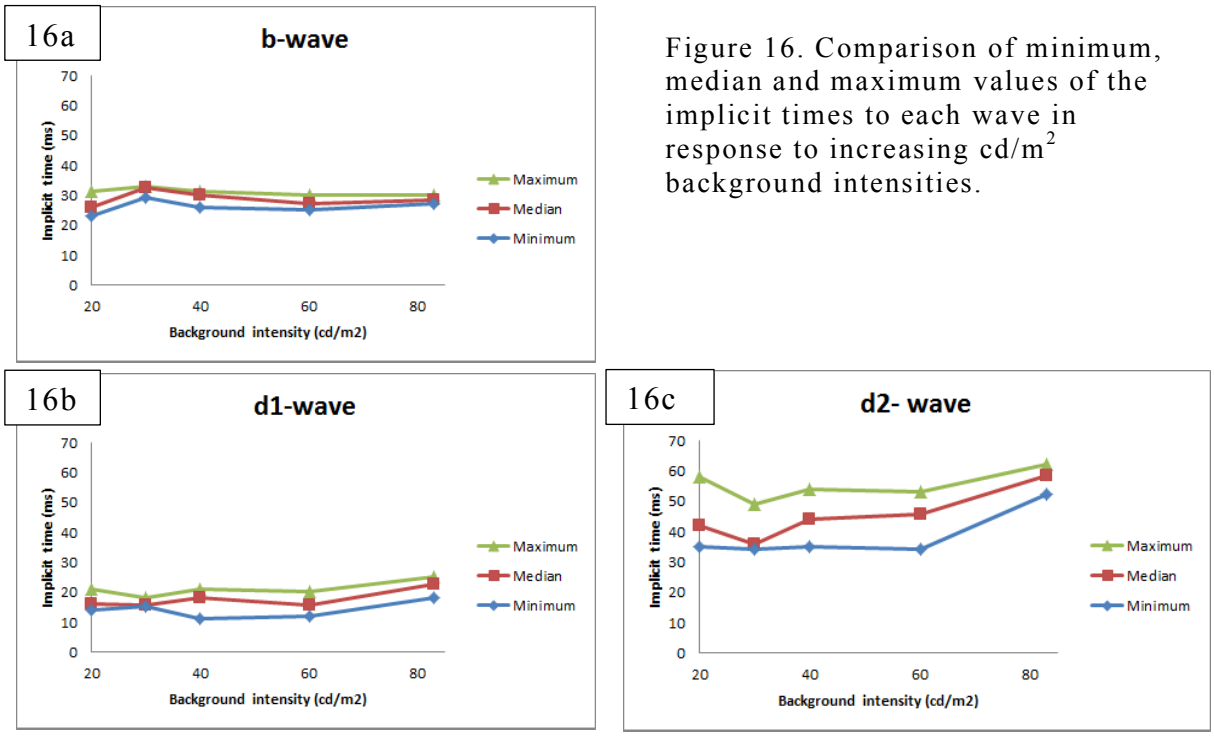


Figure 16. Comparison of minimum, median and maximum values of the implicit times to each wave in response to increasing cd/m^2 background intensities.

It was apparent that the b-wave implicit time is longer than that of the d1-wave for all intensities at all backgrounds and that the d2-waves implicit time were longest at all background and flash intensities (figure 17).

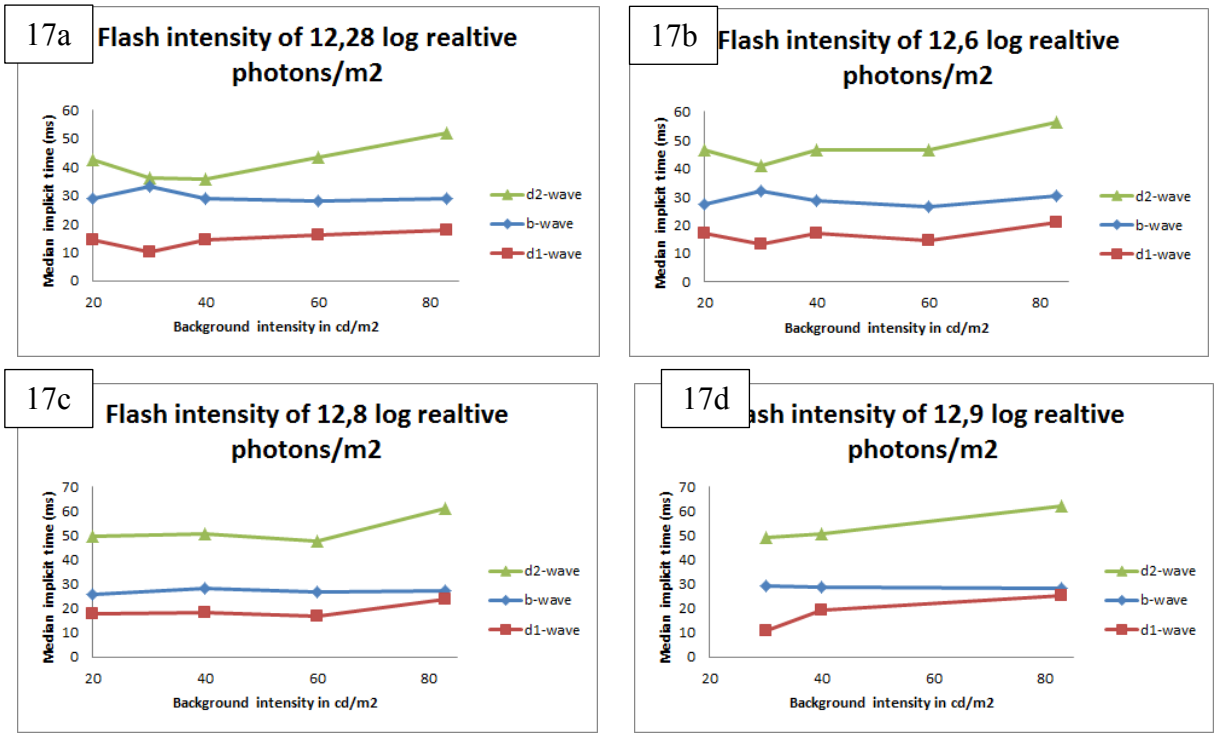


Figure 17.Comparison of b-, d1- and d2-implicit times at four different burst intensities

Using a linear regression model where implicit time was the dependant variable and background light and flash intensities were independent variables, we found that both increasing background and flash intensities increased the implicit times of the two components of the OFF-response studied, whereas significantly shorter implicit times of the b-wave were seen when the flash intensity increased. However, the coefficients of determination for our models were rather low, except for the d2-wave.

Table 3. Using a linear regression model to study the effects of background and flash intensities, respectively, on the implicit times of the b-, d1-, and d2- waves.

The sign of parameter estimates for the background light and flash intensity describe whether they increase or decrease (negative values) the implicit times.

The coefficients of determination, R^2, describe how well the model explains the measured implicit times.

Table 3 Implicit time.

b-wave

Parameter	*Parameter estimate*	*p-value*
(Intercept)	74,53	<0,0001*
Background light	0,007	0,72
Flash intensity	-3,45	0,0024*
R^2= 19%		

d1-wave

Parameter	*Parameter estimate*	*p-value*
(Intercept)	-41,076	0,0118*
Background light	0,04	0,0465*
Flash intensity	4,16	0,0008*
R^2= 28%		

d2-wave

Parameter	*Parameter estimate*	*p-value*
(Intercept)	-150,81	<0,0001*
Background light	0,15	0,0016*
Flash intensity	14	<0,0001*
R^2= 48%		

Evaluation of amplitude

Amplitudes for all curves were also compared. The median amplitudes of the b-, d1- and d2-waves at different background and flash intensities are shown in figure 18. The d1-wave was generally the smallest one in amplitude, whereas the d2-component usually had the largest amplitude. The b-wave amplitudes normally fell inbetween the d1- and d2 amplitudes.

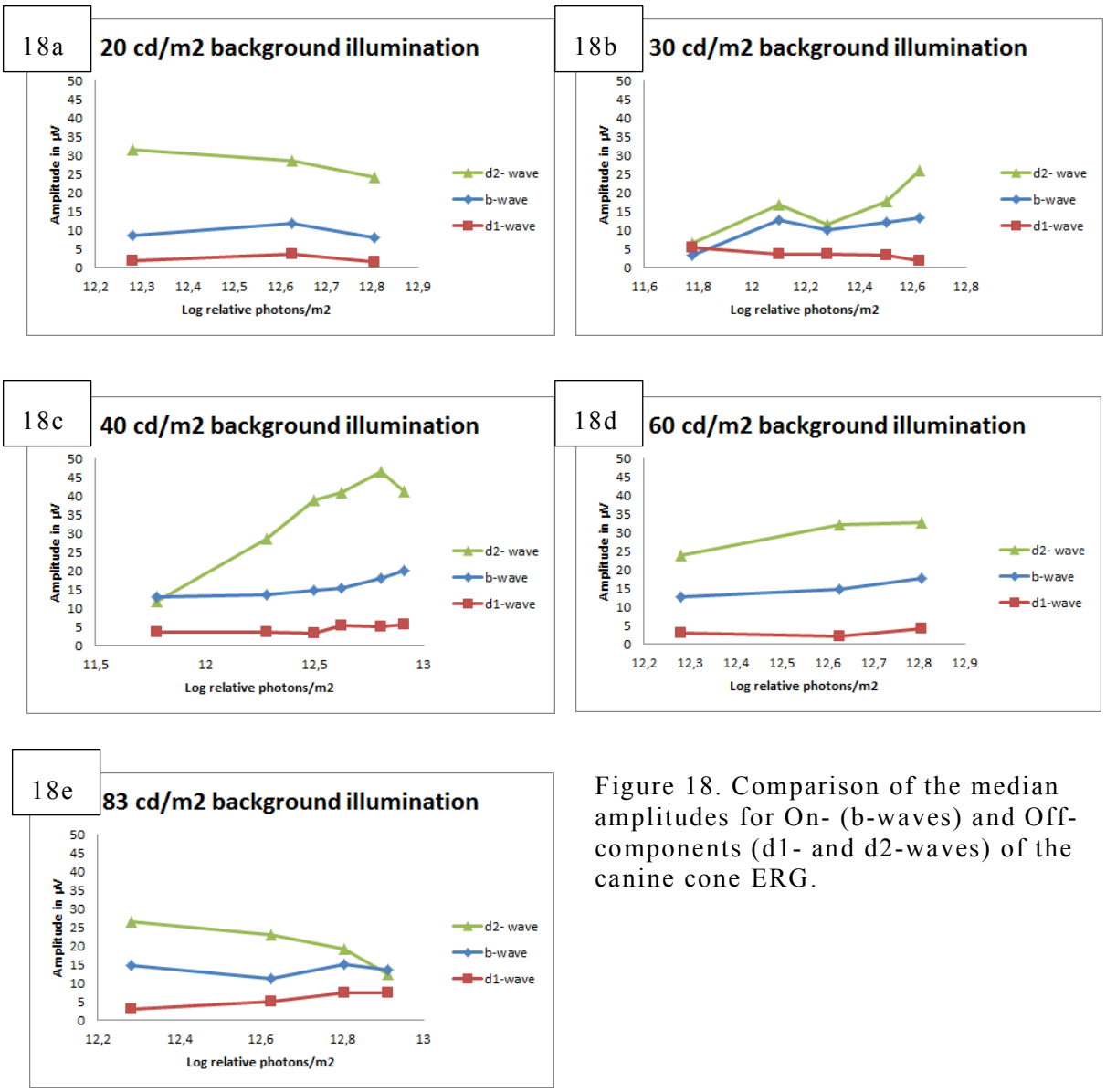


Figure 18. Comparison of the median amplitudes for On- (b-waves) and Off-components (d1- and d2-waves) of the canine cone ERG.

Similar linear regression models were used to study the effects of background and stimulus intensities on the amplitudes as for the implicit times (table 4). Interestingly, only flash intensity showed a significant positive effect on the two OFF-response parameters, whereas increases in b-wave amplitude were seen both when the background and stimulus light intensities were increased. However, R_2-values were low to moderate, probably due to rather large variation in our small material.

Table 4. Statistical analysis of the influence of the background light and flash intensities on the b-, d1- and d2-amplitudes.

A positive parameter estimate shows that they increase the amplitudes, whereas a negative value indicates the opposite.

The R^2-values shows the proportion of variability in our amplitude data that is accounted for by the model in total.

Table 4. Amplitude

b-wave

Parameter	*Parameter estimate*	*p-value*
Intercept	-53,7	0,068
Background light	0,17	0.0003*
Flash intensity	4,43	0,041*
R^2= 27%		

d1-wave

Parameter	*Parameter estimate*	*p-value*
(Intercept)	-29,32	0,0252*
Background light	-0,01	0,5196
Flash intensity	2,5	0,0104*
R^2= 14%		

d2-wave

Parameter	*Parameter estimate*	*p-value*
(Intercept)	-244	0,0004*
Background light	0,11	0,2775
Flash intensity	19,88	0,0001*
R^2= 32%		

ERG-curves; for visualization of implicit time- and amplitude changes

In figure 19 four different ERGs, all from the same dog, attained with the same flash intensity at increasing background intensities are shown. The effect on b-wave implicit time was small, whereas, d1- and d2- implicit times increase when the background light is bright.

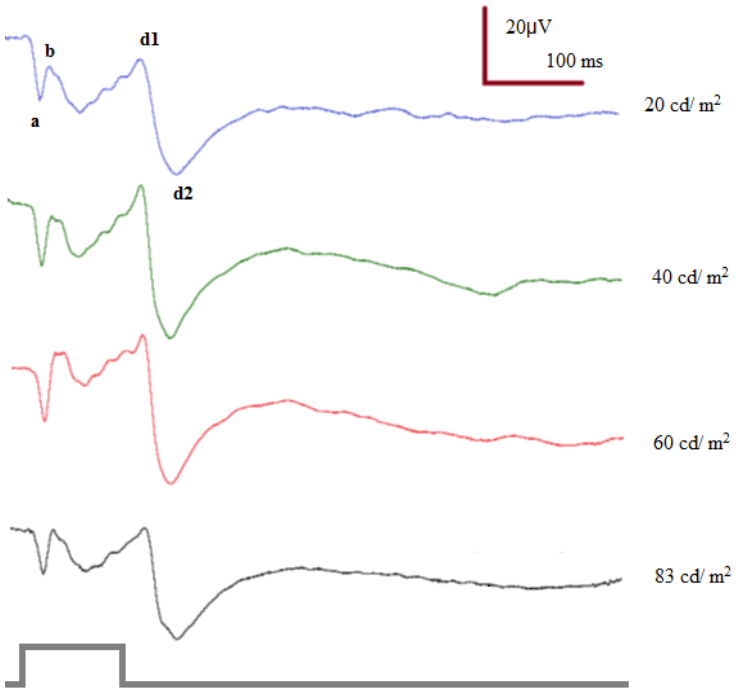


Figure 19. The effect of background illumination on implicit times. All curves are in response to a flash intensity of 12.63 log relative photons/m^2/s.

More conspicuous positive OFF-responses (d1) were seen in some experiments than others (figure 20). Despite that the overall waveform is similar in both dogs, the a-waves are deep and b-waves lower in the dog that shows the most prominent d1-waves. This may reflect individual differences, but may also show that the retinal illumination was slightly different in the two dogs in the figure due to methodological issues.

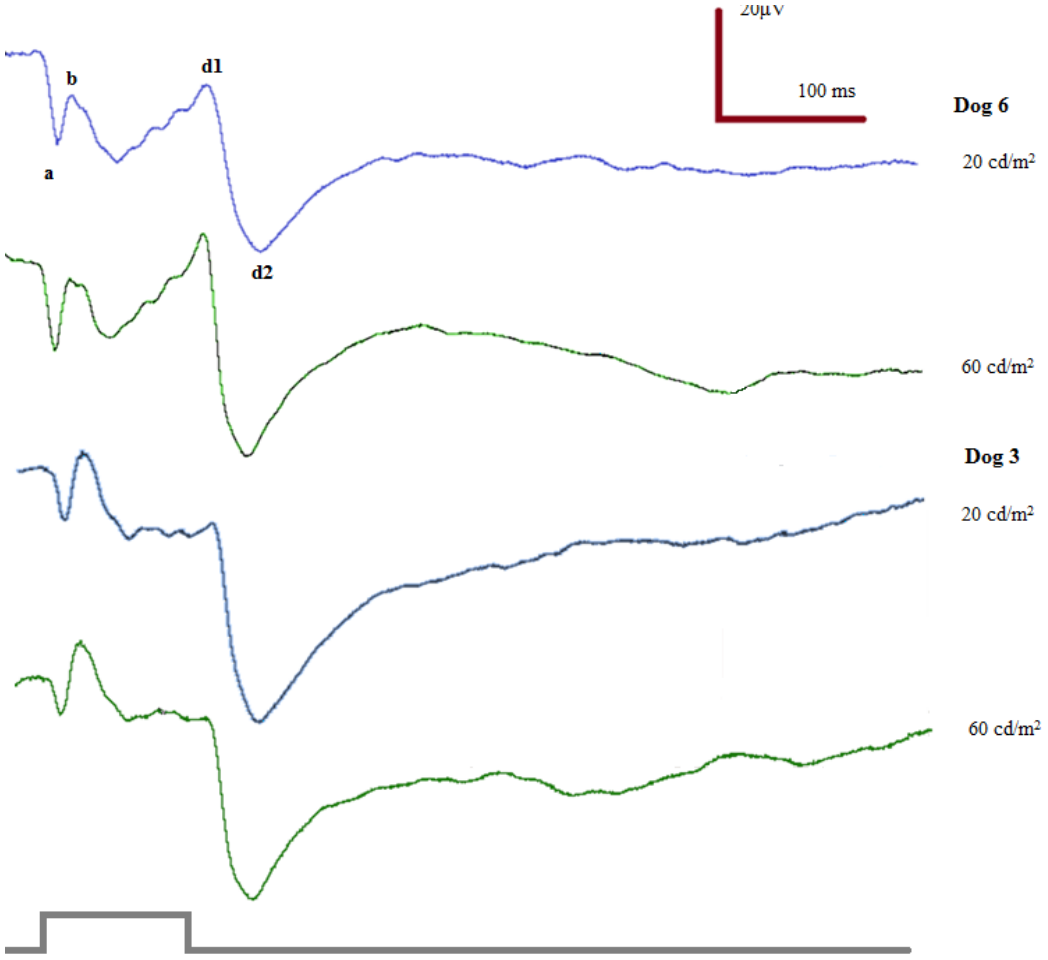


Figure 20. The individual differences to the same stimuli of 11.63 log relative photons /m^2. The upper two curves are from dog 6 at 20 and 60 cd/ m^2 background intensities. The lower two curves are from dog 3 at the same background illuminations.

The effect of increased flash intensity is illustrated in figure 21. The b-wave implicit time and amplitude were affected less than the OFF-response components in this dog. Both d1- and d2-implicit times increase when the stimulus is made brighter and the amplitudes increase, in particular the d2-amplitude.

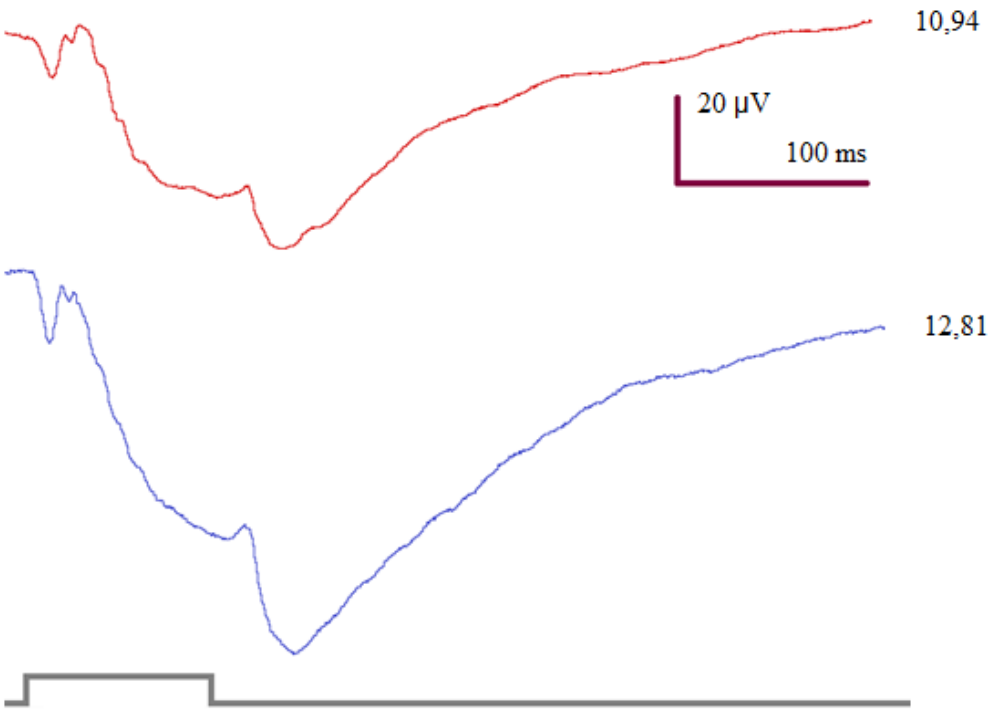


Figure 21. Effect of increased flash intensity. Two curves from the same dog recorded at a background intensity of 40 cd/m^2;

The upper curve is produced by a 10.94 log relative photons/m^2/s and the lower curve is produced by a 12.81 log relative photons/m^2/s

DISCUSSION

The presence of an OFF-complex including a positive d-wave (our d1) followed by a negative wavelet (our d2) in response to longer duration stimuli was consistent for all dogs in this experiment, which shows that a cone OFF-response is present in the dog. Sustar et al. (2006) found that a 50 ms flash duration was sufficient to produce a d-wave in the human ERG. We used different flash durations in our study and a d-wave was easily discerible when flash duration exceeded 50 ms. In our study, the dog's ERG showed a resemblance to the cat ERG that Zrenner and Gouras (1979) presented, which also contained a small, positive d-wave and a large negative OFF-response. The difference from the human d-wave is evident. The reason for this species difference is yet unknown. The fact that humans and other primates that have a similar d-wave, differ from those of cats and dogs may be partly attributed to their difference in cone to rod ratio, as well as the post-receptoral retinal circuitry.

The attained d1-wave, or positive OFF-response, is similar to the primate d-wave and its origin is most likely the same as that of the primate d-wave, which is the OFF-bipolar cells. The origin of the negative OFF-response recorded may be a combined effect of the PhNR, cone-PIII and the cessation of PII or a different neuroretinal cell response. Further studies are needed to evaluate this phenomenon and identify its cellular origin.

The regression models showed that increases in flash intensity had significant effect on both implicit times and amplitudes for all curves. An increased flash intensity increased the amplitudes of the b-, d1- and d2- waves, as well as the implicit times for the d1- and d2-waves, wheras it decreased the implicit time of the b- wave. A brighter flash thus affect both implicit time and amplitude for both ON- and OFF- responses.

The effect of increased background intensity was not completely explained by our regression models. The b-wave amplitude increased when background illumination was brighter, but the background light intensity was not shown to have a significant effect on the implicit time. Increases in background intensity affected the implicit times of the responses by a linear increase (both OFF-responses) and decrease (ON-response) in duration, however the amplitude changes could not be described by a linear regression model. Consequently the amplitude of the ON-response, as well as the implicit times for the OFF-responses, can be affected by increases in background illumination.

The results need to be interpreted with caution because data only from five dogs were included in this study. Individual differences, such as metabolic status (oxygen saturation, blood glucose level etc.), pupil diameter, direction of gaze, will have a protruding effect.

The d1-implicit time is always shorter than that of the b-wave. This may reflect the different glutamate receptors of ON- and OFF-bipolar cells. As mentioned previously, the ON-bipolar mGlu-receptors are slow, inhibitory receptors, whereas the OFF-bipolar iGluRs are fast, excitatory receptors.

The d2- wave on the other hand, is more variable in both amplitude and implicit time than the b- and d1-waves. This could possibly be an indication of a multicomponent origin, but could also reflect that measuring the d2-component correctly is more difficult because of its rounded appearance compared to the more pointed b- and d1-peaks.

The cat and the dog, both being crepuscular animals in the wild, have similar appearance of their OFF-responses in contrast to those of the diurnal human beings. The effect of this difference to human beings on canine perception is uncertain. Perhaps dogs register differences in brightness during daylight differently than humans do, but it is more likely an indication of differences in retinal wiring and signal transmission.

Separation of ON- and OFF-responses of the human ERG is often performed using a white stimulus on a white background. The blue background illumination and flash stimulation in this experiment was chosen based on Neitz and co-workers' (1989) description of the dog's dichromatic cone vision. They showed that the canine S- and M/L-cone absorption curves coincide at about 480 nm and that the dog is unable to distinguish different hues in this part of the spectrum from an achromatic stimulus. This means that both cone mechanisms will be equally stimulated in this part of the spectrum and a dog will therefore not be able to perceive different wavelengths close to 480 nm as different colors. If we compare this reasoning with a trichromatic species, such as humans, who possesses S-, M- and L-cones; a "normal white light" is a mixture of wavelengths that equally stimulates all three cone types. In our study, LEDs with a peak wavelength of 470 nm were used, which is close to the part of the spectrum

that the dog may perceive as colorless, i.e. white. The peak wavelength of our LEDs may have been a slightly better stimulus for the S-cones than the M/L-cones, but we consider that this would be of minimal consequence since the M/L- to S-cone ratio is very large (Mowat et al., 2008). Therefore, the response will still receive a larger green cone contribution because of their superiority in numbers. The obtained curves had both d-waves (d1) and negative OFF-responses (d2), which certainly show that this wavelength could be used to obtain d-waves/ OFF-responses. Furthermore, using the same type of LEDs for the background light would suppress the S- and M/L-cones almost equally and, of course, saturate the rhodopsin.

Zrenner and Gouras (1979) found that the cat did not exhibit a S-cone d-wave, but only a M/L-cone d-wave. The negative OFF-response was, however, present for both S- and M/L-cones. Because we used essentially achromatic stimuli, we have not been able to study whether the cone classes in the canine retina contribute similarly as the cat's to the d-wave and the negative OFF-response (d2) or not.

Our background intensities were measured in scotopic cd/m^2, because the purpose of the background in this experiment was to saturate the rods (and the spectral sensitivity of human and canine rhodopsin is rather similar). Photopic candelas are based on the absorption spectra of human cones and therefore not relevant for animal retinas that have different cone composition. The approximation of the number of photons emitted from the LEDs used to produce the stimuli was based on that the LEDs had an almost symmetrical distribution of wavelengths centered around their peak wavelengths. However, the photometer was not calibrated for irradiance measurements without a filter and no canine photopic filters are available. We simply used the readings, based on W/m^2 (but without a correction factor), which could be used to calculate a relative number of photons per area unit in each flash. We plan to recalibrate the photometer and re-calculate the actual numbers of photons of the different stimuli.

Further investigations on the origin of the negative OFF-response as well as the contribution from the S- and M/L-cones to the d-wave in this species are needed.

CONCLUSION

The purpose of this study was to examine if the dog ON- and OFF-responses could be separated and to further examine the nature and appearance of the OFF-response.

We found that the dog has a positive d-wave followed by a larger, negative OFF-response. The waveform is similar to that of the cat. The d-wave could be separated from the b- wave by increasing the flash duration over 50 ms. A 470 nm wavelength LED was an appropriate stimulus for both background and flash stimuli. Our small material suggests that both implicit times of the OFF-components increase when either the background or stimuli become brighter, whereas their amplitudes increase in response to increasing stimulus intensity.

REFERENSES

Bush, P.A. & Sieving, P.A. (1994). A proximal retinal component in the primate photopic ERG a-wave. Investigative Ophthalmology & Visual Science, vol. 35, pp. 635-645.

Curcio, C. A., Allen, K. A., Sloan, K. R., Lerea, C. L., Hurley, J. B. & Klock, I. B., et al. (1991). Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. Journal of Comparative Neurology, vol. 312, pp. 610-624.

Ekesten, B. (2013a). Electrodiagnostic evaluation of Vision. In: Gelatt, K.N., Gilger, B.C. & Kern, T.J. (ed.), Veterinary Ophthalmology. 5th Ed. Wiley-Blackwell, Ames, Iowa, pp.684-702.

Ekesten, B., Komaromy, A.M., Ofri, R., Petersen-Jones, S.M. & Narfstrom, K. (2013b). Guidelines for clinical electroretinography in the dog: 2012 update. Documenta Ophthalmologica 127:79–87.

Frishman, L.J. (2006). Origins of the Electroretinogram. In: Heckenlively, J.R. & Arden, G.B. (ed.), Principles and practice of clinical electrophysiology of vision. 2nd Ed. MIT press; Cambridge, Massachusetts, pp. 139-184.

Gouras, P.(1965). Saturation of the rods in rhesus monkey. Journal of the Optometrist Society of America, vol. 55, pp 86-91.

Gouras, P. (1970). Symposium on electrophysiology the association for research in vision and ophthalmology and National society for the prevention of Blindness. Investigative Ophthalmology & Visual Science , vol. 9 (8), pp. 557-569.

Gouras, P. and Zrenner, E. (1979). Blue sensitive cones of the cat produce a rodlike electroretinogram. Investigative Ophthalmology & Visual Science, vol.18 (10), pp. 1076-81.

Granit, R. (1933). The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. Journal of Physiology, vol. 77, pp 207–239.

Kolb, H. (2006). Functional Organization of the Retina. In: Heckenlively, J.R. & Arden, G.B. (ed.), Principles and practice of clinical electrophysiology of vision. 2nd Ed. MIT press; Cambridge, Massachusetts, pp. 47-64.

Linsenmeier, R.A., Mines, A.H. & Steinberg, R.H. (1983). Effects of hypoxia and hypercapnia on the light peak and Electroretinogram of the cat. Investigative Ophthalmology & Visual Science , vol. 24 (10), pp. 37-46.

Miyake, Y. (2006). Congenital Stationary Night Blindness. In: Heckenlively, J.R. & Arden, G.B. (ed.), Principles and practice of clinical electrophysiology of vision. 2nd Ed. MIT press; Cambridge, Massachusetts, pp. 829-840.

Mowat, F. M., Petersen-Jones, S. M., Williamsson, H., Williams, D. L., Luthert, P.J. Ali, R. R. & Bainbridge, J. W. (2008). Topographical characterization of cone receptors and the area centralis of the canine retina. Molecular Vision, vol. 14, pp. 2518-2527.

Narfstom, K., Wrigstad, A. & Nilsson, S-E.G. (1989). The Briard dog: a new animal model of congenital stationary night blindness. British Journal of Ophthalmology, vol. 73, pp. 750-756.

Neitz, J., Geist, T. & Jacobs, G.H. (1989). Color vision in the dog. Visual Neuroscience, vol. 3, pp. 119-125.

Neitz, M. and Neitz, J. (2000). Molecular Genetics of Color Vision and Color Vision Defects. Journal of the American Medical Association Opthalmology. Vol 118 (5), pp. 691-700.

Noell, W. K. (1952). Electrophysiologic study of the retina during metabolic impairment. American Journal of Ophthalmology, vol. 35, pp. 126-132.

Nunnery, C., Pickett J.P. & Zimmerman, K. L. (2005). Congenital stationary blindness in a Thoroughbred and a Paso Fino. Veterinary Opthalmology, vol. 8 (6), pp. 415-419.

Ofri, R. (2013). Optics and Physiology of Vision. In: Gelatt, K.N., Gilger, B.C. & Kern, T.J. (ed.), Veterinary Ophthalmology. 5th Ed. Wiley-Blackwell, Ames, Iowa, pp. 208-270.

Peachey, N.S., Alexander, K.R., Fishman, G.A. & Derlacki, D.J. (1989). Properties of the human cone system electroretinogram during light adaptation. Applied Optics, vol. 28, pp. 1145-50.

Peachey, N. S., Alexander, K. R., Derlacki, D. J. & Fishman, G.A. (1992). Light adaptation and the luminance-response function of the cone electroretinogram. Documenta Ophthalmologica, vol. 79, pp. 363-369.

Penn, R.D. & Hagins, W.A. (1969). Signal transmission along retinal rods and the origin of the a-wave. Nature, vol. 223, pp. 201-205.

Rufiange, M., Dassa, J., Dembinska, O., Koenekoop, R.K., Little, J.M., Polomeno, R.C., Dumont, M., Chembtob, S. & Lachapelle, P. (2003). The photopic ERG luminance-response function (photopic hill): method of analysis and clinical application. Vision Research, vol. 43, pp. 1405–1412.

Sandmeyer, L.S., Breaux, C.B., Archer S. & Grahn, B.H.(2007). Clinical and electroretinographic characteristics of congenital stationary blindness in the Appaloosa and the association with the leopard complex. Veterinary Opthalmology, vol. 10 (6), pp. 368-375

Sieving, P.A. (1993). Photopic ON- and OFF-pathway abnormalities in retinal dystrophies. Transactions of the American Ophthalmology Society, vol. 91, pp. 701–773.

Sieving, P.A., Murayama, K. & Naarendorp, F. (1994). Push-pull model of the primate photopic electroretinogram: A role for hyperpolarizing neurons in shaping the b-wave. Visual neuroscience, vol. 11, pp. 519-532.

Sustar, M., Hawlina, M. & Brecelj, J. (2006). ON- and OFF response of the photopic electroretinogram in relation to stimulus characteristics. Documenta Ophtalomologica, vol. 113, pp. 43-52.

Ueno, S., Kondo, M., Ueno, M., Miyata, K., Terasaki, H. & Miyake, Y. (2006). Contribution of retinal neurons to d-wave of primate photopic electroretinograms. Vision Research, vol. 46, pp. 658-664.

Wali, N. & Leguire, L. E. (1992). The photopic hill: A new phenomenon of the light adapted Electroretinogram. Documenta Ophthalmologica, vol. 80, pp. 335-342.