



## Guidelines for clinical electroretinography in the dog

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These procedures described for the dog ERG were approved at the 1st European Conference on Veterinary Visual Electrophysiology in Vienna, Austria, May 30, 2000. Dr. Narfström was Chair of the Committee for a Harmonized ERG Protocol, appointed by the European College of Veterinary Ophthalmology (ECVO), and Dr. Ofri was secretary. The other coauthors are committee members. Guidelines for ERG procedures in other animal species for clinical and laboratory studies are planned for in the future and the present guidelines are planned to be revised on a biannual basis. A brief report of the recommended procedures is available in the Conference Proceedings book.

**Key words:** clinical electrophysiology, dog electroretinography, guidelines

**Abbreviations:** ERG – electroretinography, electroretinogram, SF – standard flash

### Introduction

In dogs, as in many other animal species, subjective assessment of retinal function is not readily available. Various clinical and experimental studies have proven that the ERG is an effective and objective method of assessing retinal function [1–5]. This method has, therefore, been utilized for many years in clinical veterinary medicine (for a review see Ofri [6]) mainly for the diagnosis of retinal dystrophies and for pre-operative evaluation of retinal function in conjunction with cataract surgery. In recent years, however, it has become clear that there is a need for specific guidelines for ERG procedures in the dog in order to obtain reliable and reproducible results [7].

This document is intended as a guide to ERG procedures in canine clinical veterinary practice. Simple technical procedures will be described that permit reproducible ERGs to be obtained under specifically defined conditions. Basically two types of procedures are described. One type is a quick ERG examination that determines whether a response is present or absent and is used mainly pre-operatively before cataract surgery or in the differential dia-

gnosis of specific blinding disorders in the dog, such as in the acutely blinding disease Sudden Acquired Retinal Degeneration (SARD). The other and most important type of ERG testing described in this paper are the methods used to diagnose generalized, often hereditary outer retinal disease. These methods should be considered a minimal functional study of the retina which in no way preclude the use of additional electrophysiologic tests as each laboratory deems appropriate. We recommend that, for cross laboratory comparison, a minimum of 4 specific procedures be performed and standardized in each laboratory.

In human ophthalmology, a standard for clinical electroretinography has been recommended and up-dated three times. The latest update was approved at the International Society of Clinical Electrophysiology of Vision meeting in Eilat, Israel, April 15, 1999 [8]. Guidelines for calibration of stimulus and recording parameters used in visual clinical electrophysiology were published in 1998 [9]. Both of these documents have been used as the basis for the present publication. Although several parameters and techniques are directly comparable between the canine and human species, there are some peculiarities that have to be taken into account when working with dog patients, thereby causing the need for a specific canine protocol. One major difference between the human and dog patient is the need for anesthesia in dogs. Full surgical anesthesia is mandatory especially for doing the longer type of diagnostic ERG protocol. In the dog, which lacks the ability for subjective participation and cooperation, dark adaptation is objectively studied by successive ERG recordings, as recommended in this document.

The organization of this report consists of 3 parts. The first is a short description of the basis for the test of rod and cone function in the dog. The second part is devoted to some technical aspects of ERG, such as equipment and patient preparation. The third part consists of a summary of the two recommended protocols. The first protocol is abridged for rapid evaluation of retinal function. It is important to note that this protocol should not be used to diagnose generalized photoreceptor disease, as it does not provide a comprehensive test of specific rod and cone function. Diagnosis of generalized outer retinal diseases should be based on the second protocol, which provides more extensive evaluation of outer retinal function.

### **Testing of rod and cone function in the dog**

The rod and cone function should be precisely evaluated using separate testing procedures. Care should be taken to avoid interference between dark- and light adaptation. Both the dark- and light adaptation processes are time-consuming procedures and need to be meticulously performed. It is up to the

clinician to choose which procedure to do first as long as the recommendations below are followed for either scotopic (dark adapted) or photopic (light adapted) ERG recordings.

### *Rod function*

Rod function can be tested through studying the process of dark adaptation, by stimulating the dark-adapted retina with low levels of light and by testing the rod responses.

#### *Process of dark adaptation and dark adapted responses*

The dog is dark adapted for a minimum of 20 min. The dynamic process of dark adaptation is evaluated by periodic examination. This examination will be conducted by performing an ERG recording every 4 min during the 20-min dark adaptation period in response to low-intensity stimuli. The use of white light is recommended. Intensity of the white flash stimulus should be 2 log units below that of the standard flash (SF) that is used to test cone function (see below), or 0.02–0.03 cd/m<sup>2</sup>/sec [8]. A single flash, repeated at 4 min intervals, tests rod function and evaluates the process of dark adaptation. Averaged responses, usually not more than 4 responses, may be used and presented at a rate of 0.5 Hz or preferably less.

#### *Mixed rod and cone function*

This test consists of the response to a single high-intensity flash, 2–3 cd/m<sup>2</sup>/sec. following the dark-adaptation study. If averaging is needed not more than one flash every 10 s is recommended in order not to light adapt the rods.

#### *Cone function*

Cone function is tested by light adapting the retina with a fixed multiple (about 15 times) of the brightness of the SF in order to desensitize the rod system and then stimulating the cones with high intensity light stimuli. Dark- and/or light adapted flicker responses test the cone system as well if stimulated at a minimum frequency of 30 (or 31 Hz, see comments below).

We recommend that the dog is light adapted for 10 min using white background light with an intensity of 30–40 cd/m<sup>2</sup> [8]. The background light should be uniformly distributed across the retina using a Ganzfeld dome or similar equipment. Ambient room light is not regarded as uniform lighting for this purpose.

The function of the cones is tested by high intensity light stimuli using SF (2–3 cd/m<sup>2</sup>/sec) [8]. A single flash may occasionally be sufficient to test cone

function. If averaging is used 4 flashes or more should be presented at the rate of 4.9 or 5.1 Hz.

Cone flicker responses in the dog should be evaluated at a minimum frequency of 30 Hz, but can also be tested using higher frequencies with SF intensity. We recommend the use of 31 Hz (and, for example, 49 or 51 Hz) to avoid possible interference by mains-frequency artifactual currents, i.e. at 50 or 60 Hz, depending on region.

## **Technical aspects**

### *Patient preparation*

The preparation of the patient should be conducted in ambient light. Care should be taken to prevent pre-exposure to strong light. If fundus photography or similar exposure is required, the protocol must be adjusted accordingly and the patient dark-adapted for a longer time period than previously recommended. A dark-adaptation period of 1 hour is recommended following fundus photography or fluorescein angiography.

Sedation is insufficient for diagnostic ERG recordings (using the longer type of protocol). The dog must be fully anaesthetized in order to prevent artifacts through involuntary muscle movement. As the recording may be affected by the clinician's choice of anesthetic, the anesthesia protocol used in the recording should be reported. Note that it is important that the same anesthesia is used in the age-matched control animals, used as normal dogs in relation to the tested patient.

Proper oxygenation and ventilation must be maintained throughout the examination through intubation. Body temperature must be controlled and kept stable at 38–39 degrees centigrade. Pupils must be fully dilated throughout the examination and periodic evaluation of pupil size conducted at least at the beginning and at the end of the testing procedure.

Eyelids must be open during the examination and both corneas protected with non-irritating solution, such as 0.5% methyl cellulose [8]. Proper positioning of the pupil in relation to the stimulating light must be maintained. The use of subconjunctival stay sutures at the limbus to stabilize the globe, or other adequate means, is recommended.

### *Light stimulator and light stimulation*

The committee recommends the use of full-field conditions, such as the Ganzfeld stimulator, in order to obtain a uniform distribution of light across the retina. Ocular diffusers, such as opalescent contact lenses, are not recommended. The reason for this is that such stimulators make precise measurement

of the distribution and intensity of retinal illumination difficult. This aspect is important when evaluating generalized photoreceptor disorders of dogs, which may have a regional distribution in the fundus, sometimes sparing the central parts until late in the disease process. If any laboratory chooses to use ocular diffusers, they must independently demonstrate equivalence to full-field conditions.

Light flashes should be no more than 5 milliseconds long [8]. Uni- or bilateral stimulation may be used when testing for hereditary retinal degenerative disease, since these diseases in dogs are bilateral and at approximately the same stage of disease in both eyes.

In addition to producing uniform flashes of light, the stimulator must be able to produce an even background luminance across the fundus. Care must be taken to measure the stimulus luminance energy on the surface of the Ganzfeld sphere and not the illumination provided by the source [8]. We recommend the use of white light, both for stimulus and background in this guide. However, we recognize that colored light stimuli and/or backgrounds are also used for special purposes in some laboratories. These test conditions should be regarded as additional to the recommended standard and should not replace it.

There must be methods for modifying the light stimulus. This may be provided by using neutral density filters in front of the light source to attenuate the light.

## *Signal acquisition*

### *Electrodes*

The use of corneal contact lens electrodes with adequate curvature is recommended. Electrodes may be reused following routine cleaning and visual quality inspection. Measures must be taken to prevent drying of the corneal surface. A reference electrode, if not included in a bipolar contact lens, should be placed halfway between the temporal canthus and the ear. Commercially available electrodes can be used or subcutaneous wires, such as 0.3 mm (in diameter) silver wires, sterilized by alcohol immersion, inserted through an 18 gauge needle, are recommended as reference electrodes. A similar subcutaneous ground electrode should be placed at an indifferent location, such as at the central top portion of the head. It is recommended to evaluate electrode impedance, measured with an impedance meter. Preferably the impedance, at a frequency between 10 and 1000 Hz, should be maintained below 2 K-ohms and must be no more than 5 K-ohms [8].

### *Filters and amplifiers*

Bandpass filter setting should be as wide as possible for ERG recordings. It is recommended that the low filter (high pass) should be no higher than 1 Hz ( $-3\text{dB}$ ) and that the high filter (low pass) should be no lower than 300 Hz ( $-3\text{dB}$ ). Notch filter should be avoided.

Oscillatory potentials (OP) can be observed as small wavelets mainly on the rising phase of the b-wave using high intensity light stimulus. If a specific study of the OPs is wanted, it is recommended to use the maximum scotopic rod and cone response obtained after the single SF of white light and filter the response at the end of the ERG testing procedure using a low filter setting at 70–100 Hz.

Equipment should enable amplification of the signal so that recordings may be evaluated with high accuracy (10 000–20 000 times for standard flash ERGs and 100 000 for OPs). It is recommended that the equipment meets EU safety standards for (human) clinical ERG.

### *Reporting of results*

Reports in the literature should include a display of the dog's ERG traces alongside the traces of a normal, age-matched dog of the same breed, anaesthetized using the same anaesthetic protocol. Calibration bars should be added. It is recommended that a pre-stimulus baseline, as well as an indication for the onset of light stimulus is presented. The duration of the recorded response or sweep time should routinely be 200 milliseconds. A report of the ERG recording should include the following parameters:

1. a-wave amplitude: Measured from the baseline to the a-wave trough
2. b-wave amplitude: Measured from the a-wave trough to the b-wave peak
3. a- and b-wave implicit time: Measured from the stimulus onset to the a-wave trough and b-wave peak, respectively
4. An illustration of the dark-adaptation curve, such as plotting the amplitude on the ordinate and dark adaptation time on the abscissa, including normal limits for a dog of similar breed, similarly anaesthetized and of similar age.

It is, therefore, important that each laboratory performing diagnostic ERGs for generalized retinal diseases obtain their own normal values using their ERG equipment and for the specific breeds of dogs routinely studied as well as normal values for the main age-groups studied. The reason for this is that dog ERGs vary as a function of many factors such as anesthesia type and level, age and the position of the reference electrode. The resistance and, therefore, the voltage of the ERG signal varies especially widely in dogs when a skin reference is used because of the large variation in skull size and depth of intervening bone between breeds. We recommend that reports of results

include the normal values for the specific breed and age-group, preferably indicating the median values and the limits of normality [11] using the 5th and 95th percentiles.

## Summarized protocols

### *Recommended short protocol for gross retinal function*

The short protocol is intended to rapidly determine gross retinal function in dogs that are about to undergo cataract surgery or, for instance, in which the diagnosis of retinal versus central blindness is to be evaluated. It is NOT an adequate test of rod and cone function in patients that may be suffering from inherited photoreceptor dysplasias or degenerations. If any generalized photoreceptor anomaly is suspected, the longer diagnostic protocol for specific photoreceptor evaluation should be used.

The dog is prepared in ambient light.

1. Test retinal function in ambient light using the SF.
2. Turn off the light and test retinal function within the first minute of dark adaptation using SF.
3. Test retinal function again after 5 min of dark adaptation using SF.

### *Recommended protocol for diagnosis of photoreceptor disorders*

This protocol is intended to test for generalized and most often inherited photoreceptor disorders of dogs. For a summary of affected breeds and their respective diseases as well as a timetable when ERGs are considered diagnostic, see Table 1.

The dog is prepared in ambient light after which the light is turned off.

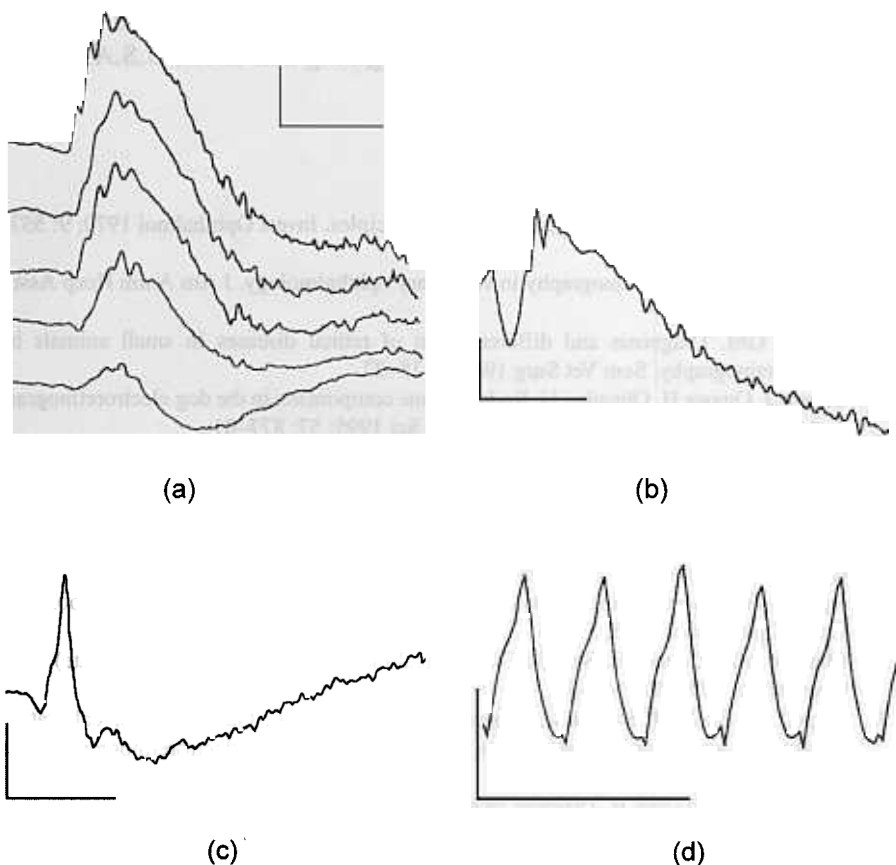
1. Dark adapt for 20 min while evaluating rod function and the dynamic process of dark adaptation every 4 min (at 1, 4, 8, 12, 16 and 20 min).
2. Test the mixed rod and cone response.
3. Test the cone function after 10 min of light adaptation.
4. Perform the cone flicker test.

For an illustration of representative ERG responses obtained using this protocol see Figure 1A–D.

*Table 1.* Some hereditary retinal diseases in dogs. For further information and references regarding these diseases, see *Veterinary Ophthalmology* 3rd edition [10]

Affected structure(s)	Breed	Disease / Gene symbol	Mode of inheritance	Diagnosis by ophthalmoscopy	Diagnosis by ERG
neuroretina	Alaskan Malamute	cone degeneration (hemeralopia)	AR		6 w
neuroretina	Toy and Miniature Poodle	progressive rod cone degen. / prcd	AR	3–5 yrs	9 mo
neuroretina	Labrador Retriever	progressive rod cone degen. / prcd	AR	3–6 yrs	1.5 yrs
neuroretina	American Cocker Spaniel	progressive rod cone degen. / prcd	AR	2.5–3 yrs	9 mo
neuroretina	English Cocker Spaniel	progressive rod cone degen. / prcd	AR	3–8 yrs	2–3 yrs
neuroretina	Portugese waterdog	progressive rod cone degen. / prcd	AR	3–6 yrs	1.5 yrs
neuroretina	Akita inu	progressive retinal atrophy	AR	1–3 yrs	1.5–2 yrs
neuroretina	Longhaired Dachshund	progressive retinal atrophy	AR	6 mo	4 mo
neuroretina	Papillon	progressive retinal atrophy	AR	1.2–5 yrs	9 mo–1.5 yrs
neuroretina	Tibetan Spaniel	progressive retinal atrophy	AR	3–5 yrs	1.5 yrs
neuroretina	Tibetan Terrier	progressive retinal atrophy	AR	1–1.5 yrs	10 mo
neuroretina	Irish Setter	rod dysplasia type 1 / rcd1	AR	16 w	6 w
neuroretina	Collie	rod dysplasia type 2 / rcd2	AR	16 w	6 w
neuroretina	Norwegian Elkhound	rod dysplasia / rd	AR	1–1.5 yrs	
neuroretina	Norwegian Elkhound	early rod degeneration / erd	AR	9 mo–1 yr	5 w
neuroretina	Siberian Husky	X-linked progressive retinal degeneration / XL PRA	X-linked	2 yrs	1 yr
neuroretina	Miniature Schnauzer	photoreceptor degeneration / pd	AR	1.5–5 yrs	
RPE + neuroretina	Briard	congenital retinal dystrophy, RPE65 defect	AR	4–6 yrs	5 w

Symbols: AR autosomal recessive, AD autosomal dominant.



**Figure 1.** (a–d) Example of ERG responses obtained from a 2-year-old mixed breed, middle sized dog recorded at the Section for Ophthalmology, Faculty of Veterinary Medicine, Uppsala, Sweden, using the currently presented protocol for diagnosis of photoreceptor disorders. In these recordings, the anesthesia was induced using thiopental sodium and maintained by isoflurane by an endotracheal tube. The flash stimulus has not been indicated in these figures since the stimulus initiates the ERG recording. Calibration symbol is depicted at the side of the recordings. (a) Single flash rod responses during dark adaptation here shown at 1, 5, 10, 15 and 20 min, respectively, from bottom to top. Calibration: Horizontal: 50 milliseconds, Vertical: 20 microvolts. (b) Single flash dark-adapted combined rod and cone response. Calibration: Horizontal: 50 milliseconds, Vertical: 60 microvolts. (c) Single flash light adapted cone response. Calibration: Horizontal: 50 milliseconds Vertical: 4 microvolts. (d) 50,1 Hz cone flicker recording. Calibration: Horizontal: 50 milliseconds, Vertical: 5 microvolts.

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