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ISCEV STANDARDS

Guidelines for clinical electroretinography in the dog: 2012 update

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Abstract The full-field, flash electroretinogram (ERG) is now a widely used test of canine retinal function for the clinical diagnosis of hereditary retinal dystrophies and other causes of retinal degeneration, assessment of retinal function in patients with opaque media, ruling out of generalized retinal diseases in patients with sudden loss of vision and in ophthalmological research, as well as in pharmaceutical and toxicological screening for deleterious side effects of drugs and other chemical compounds. In 2002, the first guidelines for clinical ERGs in this species adopted by the European College of Veterinary Ophthalmologists were published. This work provides an update of these guidelines.

Keywords Canine · Standard · Protocol · ERG · Rods · Cones · Retina

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Introduction

In dogs, as in most other animal species, subjective assessment of retinal function is difficult even for the experienced clinician. Thus, for more than a century, recording of electrical currents driven by the photoreceptors in response to light stimuli has been used to objectively assess retinal function in the dog [1]. The full-field, flash electroretinogram (ERG) is now a well-established test of canine retinal function for the clinical diagnosis of hereditary retinal dystrophies and other causes of retinal degeneration. ERG is also used to assess retinal function in patients with opaque media and rule out generalized retinal diseases in patients with sudden loss of vision. Furthermore, it is an important tool in ophthalmological research and in pharmaceutical and toxicological screening for deleterious side effects of drugs and other chemical compounds.

The canine retina is dominated by rod photoreceptors. The spectral sensitivity of the rhodopsin is similar to what is seen in other species and peaks at 495 nm [2]. Two types of cones have been identified, one most

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sensitive to middle- to long- wavelength light (555 nm) and a less common cone type that is maximally sensitive to short-wavelength light (429 nm) (see [3] for review). Both cone types are spread ubiquitously over the retina and their density is higher in the horizontal visual streak and peaks in the area centralis temporal to the optic nerve head.

The need for clinical guidelines, in analogy to the human ERG standards issued by the international society for clinical electrophysiology of vision (ISCEV), led to a draft protocol for clinical electroretinography in the dog that was presented at the first European Conference on Veterinary Visual Electrophysiology in Vienna, Austria, in 2000. Two years later, the first guidelines for clinical ERGs in this species adopted by the European College of Veterinary Ophthalmologists were published [4]. This work provides an update of the canine guidelines published in 2002, and the authors strongly recommend that anyone using ERG as a diagnostic tool in dogs adheres to these new guidelines.

In this update, we define stimulus and background light intensities more precisely to improve the consistency of ERG responses recorded using this protocol and to facilitate comparison of findings between different clinics and laboratories. We have used the word "intensity" in the guidelines instead of the physically correct terms "luminance over time" and "luminance" when we talk about the amount of light in the light stimulus and background light, respectively, to make the text more legible. We also use the units cd s m^{-2} and cd m^{-2} for stimulus and background intensity, respectively, despite that candela is scaled for the human and not canine visual system. We also emphasize the importance of consistent positioning of the reference electrodes and the pre-ERG light exposure of the animal.

Furthermore, we outline two optional add-ons to the protocol: a dark-adapted intensity–response series that will provide more information about the retina's ability to respond over a larger range of stimuli intensities and a dark-adapted response to a high-intensity stimulus that will bring out the a-wave more consistently. We consider both these add-ons as particularly useful for the assessment of rod-driven function in dogs where these responses are abnormal, but not completely lost.

Finally, the concept of a very brief protocol intended solely to decide whether any retinal function is present or not, for instance before cataract surgery, has been abandoned. It is up to the clinician to decide which tests to use to establish that retinal function is completely lost, and the surgeon should decide what criteria should be used for deciding whether elective surgery should be performed. The information in this article about patient preparation, equipment and settings, as well as the tests included in this updated protocol, may provide guidance to anyone wishing to assess canine retinal function using any ERG protocol.

These updated guidelines have three main sections:

- A short description of the basis for the test of rod and cone function in the dog.
- Some technical aspects of the recording, such as equipment and patient preparation.
- A summary of the recommended protocol and the optional add-ons.

Testing rod and cone function in the dog

Rod and cone function should be evaluated as precisely and comprehensively as possible using a series of dark-adapted tests for rod-driven retinal function and a set of light-adapted tests for assessing the cone-driven responses (see [5] for review). Furthermore, a dark-adapted, mixed rod–cone response is included in the recommended protocol. Examining retinal function during both the dark- and light-adaptation processes is a time-consuming procedure that needs to be meticulously performed. It is possible to start the examination either with the scotopic (dark-adapted) or photopic (light-adapted) responses, but the order of dark- and light adaptation should be consistent. However, to facilitate comparison of results between different clinics and laboratories, we recommend that the ERG examination always begins with the dark-adapted responses followed by the light-adapted responses. We have also added brief descriptions of the two optional add-ons.

It is important to remember that the contributions of the rod and cone systems to the responses will be affected if the retina is exposed to either more or less light than normal before and during the examination. Hence, fundus photography or exposure to bright sunlight just prior to the ERG examination, stray light leaking into the ERG room, excessive light stimulation during the dark-adaptation process, dimming or

turning off the room lights for a variable time before the ERG is performed, poorly dilated pupils, deteriorating sources of stimulus or background lights, etc., will affect the ERGs obtained and make them less reliable and reproducible.

Rod-driven responses during dark adaptation

Rod function may be investigated by studying the changes in ERG responses during the period of dark adaptation, using a low flash intensity to favor rod-driven responses [6, 7].

The dynamic process of dark adaptation of the dog's retina is evaluated by recording the rod-driven responses every 4 min during at least 20 min. Ideally, the process of dark adaptation should be monitored until the b-wave amplitudes in each dog have reached a stable plateau, but that would prolong the duration of the examination and anesthesia in some patients. The test is conducted in complete darkness, though a dim darkroom-like red light may be used briefly when necessary. A brief, low-intensity, white light stimulus is recommended. The intensity of the stimulus should be 0.01 or 0.02 cd s m^{-2}, which is at the lower end of the range recommended in the 2002 guidelines [4]. The reason for lowering the intensity is to reduce cone-driven contamination of the dark-adapted rod responses. We anticipate that future updates may advocate a single stimulus intensity, 0.01 cd s m^{-2}. A single flash of light is preferred, but often the recording conditions require averaged responses to reduce the amount of artifacts and noise that may otherwise distort or obscure the currents generated by the retina. Responses to up to 4 flashes, presented at 0.2 Hz, may be averaged. If single responses are obtained, it is recommended that the stimulus be repeated once using the same settings to prove reproducibility.

Optional dark-adapted intensity/response series

This is an optional test, which is not included in the recommended standard protocol. However, if the examiner decides to conduct this test, it should be performed after the 20-minute period of dark adaptation.

The dark-adapted retina is tested with a series of stimuli of increasing intensity over at least a 3-log unit range, starting with the dimmest stimulus, which should be at least 1 log unit dimmer than the stimulus used for obtaining rod-driven responses during the dark-adaptation process (that is a starting intensity of $\leq$0.001 or $\leq$0.002 cd s m^{-2}). Normally, the b-wave should not be detectable using the dimmest stimulus. The intensity should be progressively increased, in at least 7 steps, reaching an intensity of at least 3.0 cd s m^{-2}, which is the intensity recommended for the dark-adapted, mixed rod–cone response.

If averaging is needed, the number of stimuli should be kept to a minimum, in particular for the brighter stimuli, because each stimulus is likely to affect the dark-adapted state of the retina. A good way to make sure that the interstimulus delay is sufficient after averaging responses to brighter stimuli is to repeat one of the dim intensities used in the early stages of the intensity/response series after using the bright stimulus. The result of the repeated dim intensity–response should then be very similar to the original response obtained with the same stimulus intensity.

Dark-adapted, mixed rod–cone response

This dark-adapted test consists of the response to a single, bright stimulus, 3 cd s m^{-2} following the dark-adaptation study of the recommended protocol. If averaging is needed, the number of stimuli should be kept to a minimum, because each stimulus is likely to affect the dark-adapted state of the retina. Hence, a long interval between the stimuli, at least 15 s, is advocated in order to reduce the light adaptation of the retina. If single responses are obtained, it is recommended that the stimulus be repeated once to prove reproducibility.

Optional high-intensity dark-adapted mixed rod–cone response

This is an optional test, which is not included in the recommended standard protocol. However, if this test is chosen to be added by the examiner, we recommend that it be performed after the 3.0 cd s m^{-2} dark-adapted, mixed rod–cone function.

A single, high-intensity stimulus of 10 cd s m^{-2} is employed after the dark-adapted 3.0 cd s m^{-2} mixed rod–cone response. Averaging should be avoided unless very long intervals (several minutes) are used between the flashes, because of the potential for light adapting the retina when using brighter flashes.

Light-adapted cone function

Cone function is tested after light adapting the retina in order to suppress the rod system. We recommend that the dog be light-adapted for 10 min using white background light of 30 cd m^{-2}.

The function of the cones is tested using a flash of 3 cd s m^{-2}, presented on the white light-adapting background of 30 cd m^{-2}. Often averaging of responses is required to reduce artifacts and noise sufficiently, because the amplitudes are normally lower than dark-adapted rod and mixed rod–cone responses. If averaging is used, the stimulus frequency should be $\leq$5 Hz.

Cone flicker responses in the dog should be evaluated at a frequency of 30 $\pm$ 1 Hz using a flash of 3 cd s m^{-2} presented on the white, light-adapting background of 30 cd m^{-2}. The best way to obtain reliable and stable responses to the flickering stimulus is to discard the response to the first flash, which will be a single-flash cone response that interferes with the waveform of the subsequent flicker responses. Furthermore, avoiding a stimulus frequency that is a multiple of the frequency of the electrical power system may reduce the amount of noise considerably. For instance, 31 Hz stimulation may provide a cleaner flicker response in countries using 60 Hz A.C. in the power system.

Technical aspects

Patient preparation

The preparation of the patient should be conducted in normal, ambient room light. The intensity of the lights where dogs are kept before and during preparation should be equal for both clinical patients and normal control dogs, to reduce the variation in retinal adaptation. Care should be taken to prevent pre-exposure to bright lights. We recommend a recovery period of no less than 60 min in normal room illumination before the ERG recording after a regular ophthalmic examination including indirect ophthalmoscopy or after fundus photography to avoid interference caused by exposure of the retina to the lights from these instruments [8].

Sedation is insufficient for the recommended ERG protocol. The dog must be fully anaesthetized in order to prevent artifacts due to 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. The anesthesia protocol should provide a stable depth of anesthesia throughout the ERG examination. It is important that the same anesthesia be used in the age-matched control animals, and it should be remembered that anesthetic agents, such as halothane, may affect the ERG markedly or certain components (see [5] for review).

Proper oxygenation and ventilation must be maintained throughout the examination through intubation. Body temperature must be controlled and kept stable at 38 $\pm$ 1 °C. Pupils must be fully dilated throughout the examination, and evaluation of pupil size should be conducted at least at the beginning and at the end of the ERG examination. Monitoring pupil size, active electrode position, etc., during the examination should be performed using a dim, darkroom-like red light.

Eyelids must be open during the examination and both corneas kept moist throughout the examination with artificial tears, if contact lens electrodes are not used. Proper positioning of the pupil in relation to the stimulating light must be maintained, which may require use of subconjunctival stay sutures at the limbus to stabilize the globe, or other adequate means.

Light diffusion

Stimulating photoreceptors in all parts of the retina with the same amount of light is essential for generating reliable and comprehensive responses. Hence, light sources providing only focal or multifocal illumination of the retina should not be used for the stimuli or background lights.

We recommend the use of full-field stimulators (such as a Ganzfeld or mini-Ganzfeld stimulus) in order to obtain a uniform distribution of light across the retina. In addition to generating uniform flashes of light, the stimulator must be able to generate an even and steady background luminance across the fundus during light adaptation and the recording of cone responses. Ambient room light is not regarded as uniform lighting for this purpose.

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 is important when evaluating generalized

photoreceptor disorders of dogs, which may have a regional variation 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.

Unilateral stimulation may be used when testing for hereditary retinal degenerative disease, such as "classical" progressive retinal atrophy (PRA), since these diseases in dogs are bilateral and progress at a similar rate in both eyes. However, bilateral recordings that produce similar test results from both eyes may provide better support for a specific diagnosis. Furthermore, bilateral ERGs may be advantageous both for detecting unilateral conditions and diseases affecting both eyes nonsymmetrically. Normally, bilateral recordings are performed using a system allowing stimulation and recording from both eyes simultaneously, because of the difficulties to obtain identical retinal adaptation if the eyes are tested sequentially.

Stimulus and background wavelengths

We recommend the use of white light, both for stimulus and background. White light produced by a combination of narrow band sources, such as colored light-emitting diodes (LEDs), may not be equivalent to broad-band white light as a stimulus in the dog, although both may appear white to a human observer. Hence, comparison of results between normal control dogs and patients is only to be performed when the same type of light sources has been used to stimulate the retina in both groups of dogs.

We recognize that chromatic light stimuli and/or backgrounds are also used for special purposes in some laboratories. These test conditions should be regarded as supplements to the guidelines provided in this article and should not replace it.

Stimulus and background light duration

Light flashes should be no more than 5 ms long, not to exceed the integration time of the photoreceptors [4, 9].

Stimulus and background light intensities

The adapting background light source should provide a steady light throughout the process of light adaptation and during the light-adapted tests included in the recommended protocol. It is imperative that the amount of stimulus and background light reaching the cornea be equal in all dogs. Hence, we suggest that the luminance of the full-field stimulator be calibrated at least every 6 months [10], unless the manufacturer has made other recommendations. Additional calibrations should be performed if there are any indications in the ERG data suggesting that either the stimulus or background light intensities deviate from the recommended levels. A photometer that meets international standards for photometric measurements should be used to assess the stimulus and the background lights.

The intensity of both the stimulus and the background lights should be measured at the level of the patient's cornea, which implies that the light measured is the light reflected off the walls of the full-field stimulator, not the light emitted by the light source. The intensity of the stimulus is the integrated luminance over time in cd s m^{-2}. The luminance in cd m^{-2} is used when the background light is measured.

It should be possible to adjust both the stimulus and background light intensities to desired values without altering the color of the light.

In these updated guidelines, we specify both the stimulus and background light intensities to reduce the variation in the responses included in the recommended protocol (Table 1). However, a small amount of variability has to be accepted, and therefore, the intensities should fall within ±10 % of the recommended values.

Signal acquisition

Electrodes

For active electrodes, the use of corneal contact lens electrodes with adequate curvature is recommended. Electrodes may be reused following routine cleaning and visual quality inspection under at least 10 times magnification. Measures must be taken to prevent drying of the corneal surface if contact lens electrodes are not used. It should be remembered that the type of active electrode used often affects the ERGs obtained; for instance, different types of corneal contact lens electrodes may have a major impact on the responses obtained [11]. Hence, the same type of active electrode should be used in both patients and control dogs. When a corneal contact lens electrode is used, the space between the cornea and the lens should be completely

Table 1 Intensities of the stimulating and background lights for the recommended protocol and the optional add-ons (marked with asterisks and in italics)

Response	Light stimulus (cd s m^{-2})	Adapting background light (cd m^{-2})
DA rod-driven	0.01 or 0.02	No
*Intensity/response series**	*0.001 or 0.002 ≤ x ≥ 3.0*	*No*
DA mixed rod–cone	3.0	No
*DA very high-intensity flash**	*10.0*	*No*
LA cone-driven	3.0	30
LA 30 Hz flicker	3.0	30

DA dark-adapted, *LA* light-adapted

filled with a nonirritating, ionic solution that is not more viscous than 0.5 % methylcellulose solution [9] (Fig. 1).

A reference electrode should be placed at the same distance from the temporal orbital rim in all patients, usually 3–5 cm from the lateral canthus [11]. Commercially available electrodes are recommended as reference electrodes (Fig. 2). A similar electrode type should be placed at an indifferent location, usually in the midline on the top of the skull, and serve as a ground electrode. It may reduce interference if the ground electrode is positioned with approximately equal distances to the reference and active electrodes when bilateral recordings are performed.

Fig. 1 Three examples of electrodes that can be used as ground and reference electrodes in the dog. The electrode to the *left* is a button-type cutaneous electrode. The cup is filled with a sticky contact paste, which makes contact with the skin and keeps the electrode in position. The electrodes in the *middle* and to the *right* both have needles that are inserted subcutaneously (*middle*: straight metal needle; *right*: cork screw-shaped stainless steel needle)

It is recommended that the electrode impedance be evaluated with an impedance meter. The impedance, at a frequency between 10 and 1,000 Hz, should be no more than 5 kΩ [4, 10]. When bilateral ERG recordings are performed, the impedance should be as similar as possible between the electrodes on the left and right sides.

Filters

Band-pass filter settings should be as wide as possible for ERG recordings. It is recommended that the low filter (high pass) should be no higher than 0.3 Hz and that the high filter (low pass) should be no lower than 300 Hz. We are aware that some older commercial ERG machines may not be compatible with the high-pass filter setting recommended here. This equipment can still be used, but it should be stated in the reports that a different high-pass filter setting was used. Use of notch filters (a type of band-stop filter used to reduce 50 or 60 Hz noise from the electrical power system) should be avoided, because they are likely to block some of the biological signals of interest as well.

Oscillatory potentials (OPs) 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 required, it is recommended that a second dark-adapted, mixed rod–cone response be recorded using a high-pass filter setting at 70–100 Hz 15 s after the dark-adapted, 3.0 cd s m^{-2}, mixed rod–cone response.

Amplification and display of data

Equipment should enable amplification of the signal so that recordings may be evaluated with high accuracy (10^4 times for a-and b-waves, and 10^5 for OPs). A sampling rate of at least 1 kHz on each channel is recommended to avoid loss of pertinent information.

It should be possible for the examiner to view the waveforms directly during the examination and to monitor them for signs of instability or other technical problems. If single responses are obtained, it is recommended that the stimulus be repeated once to prove reproducibility.

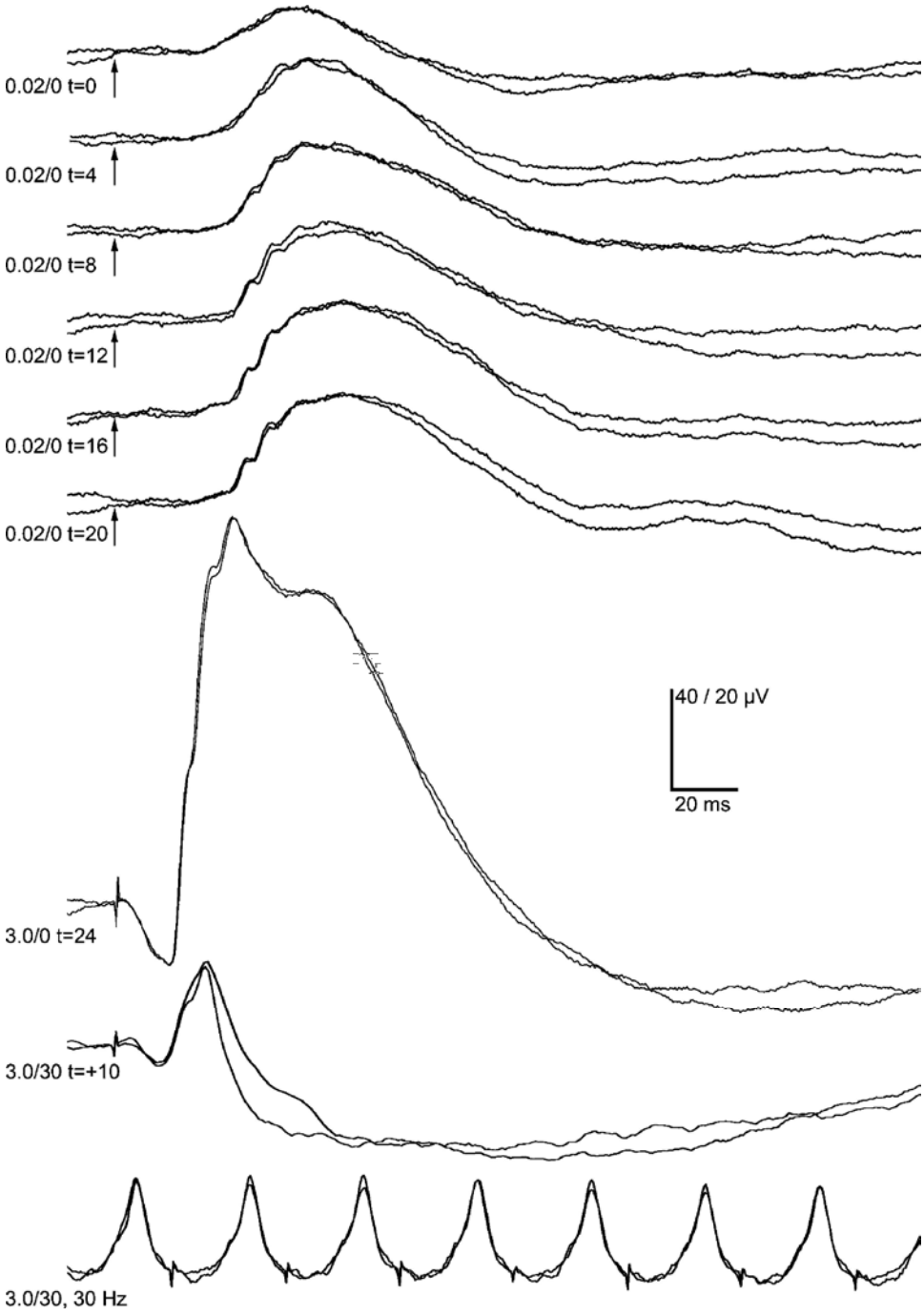

Fig. 2 ERG responses from a normal 3-year-old dog. **a** Rod-driven responses during dark adaptation at 0, 4, 8, 12, 16 and 20 min, respectively, from *bottom* to *top*. **b** Single-flash dark-adapted mixed rod–cone response. **c** Light-adapted cone response. **d** 30 Hz light-adapted flicker. The onset of the flash stimulus is indicated by *arrows*. Calibrations for the *dark*- and *light*-adapted amplitudes, respectively, and their common time scale are shown in the *middle* of the figure

The equipment should meet current applicable safety standards of clinical biological recording systems.

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 anesthetic protocol. Calibration bars should be added. It is recommended that a pre-stimulus baseline, as well as an indication of the onset of light stimulus, be presented. The duration of the recorded response or sweep time should routinely be at least 200 ms after the onset of the stimulus. 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. Amplitude of the cone flicker response, which is calculated as the average of the amplitudes from the trough to the peak in at least 3 responses in the train,
4. a- and b-wave implicit times: measured from the stimulus onset to the a-wave trough and b-wave peak, respectively,
5. Implicit time of the cone flicker response, which is calculated as the average of the times from the onset of the stimulus to the peak in at least 3 responses in the train,
6. 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 or group of dogs of similar breed, similarly anaesthetized and of similar age.

It is important that each clinic or laboratory performing diagnostic ERGs for generalized retinal diseases obtains their own normal values using their ERG equipment and establishes normal baseline values for the breeds and for the main age-groups studied. The reason for this is that dog ERGs vary as a function of many factors, including anesthesia type and level, age, the position of the reference electrode, etc., as reviewed in [5]. Breed is another important variable, as the resistance and therefore the voltage of the ERG signal vary widely because of the large variation in skull conformation between breeds. We recommend that reports of results include the normal values for the specific breed and age-group, preferably indicating the median and limits of normality using the 5th and 95th percentiles.

The recommended protocol and the optional add-ons in summary

The dog is prepared in normal, ambient room 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 (for practical reasons, the first flash (at 0 min) may be delivered after 10 s of dark adaptation and the subsequent flashes after 4, 8, 12, 16 and 20 min; Fig. 2). Flash intensity preferably 0.01 cd s m^{-2} or 0.02 cd s m^{-2}.

Optional: Perform the dark-adapted intensity/response series. Flash intensity increasing in at least 7 steps from below b-wave threshold ($\leq$0.001 or $\leq$0.002 cd s m^{-2}) to $\geq$3 cd s m^{-2}.

2. Test the mixed rod–cone response to a 3.0 cd s m^{-2} flash in the dark.

Optional: Perform the dark-adapted, high-intensity flash test. Flash intensity 10 cd s m^{-2}.

3. Test the cone function after 10 min of light adaptation (background light at 30 cd m^{-2}). Flash intensity of 3 cd s m^{-2}.
4. Perform the 30 Hz flicker test at 3 cd s m^{-2} with a rod suppressing background light of 30 cd m^{-2}.

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