Assessment of the dark-adaptation time required for recovery of electroretinographic responses in dogs after fundus photography and indirect ophthalmoscopy

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Objective—To investigate the duration of dark-adaptation time required for recovery of electroretinographic responses after fundus photography or indirect ophthalmoscopy in dogs.

Animals—6 dogs.

Procedure—Initially, scotopic-intensity series of electroretinograms (ERGs) were recorded after 20 minutes of dark adaptation. The fundus of the left eye of each dog was photographed (n = 10) or examined via indirect ophthalmoscopy for 5 minutes with moderate- (117 candela [cd]/m^2) or bright-intensity (1,693 cd/m^2) light; ERGs were repeated after a further 20 or 60 minutes of dark adaptation (6 procedures/dog).

Results—Following 20 minutes of dark adaptation after fundus photography, the b- and a-wave amplitudes were reduced in response to brighter stimuli, compared with pretest ERGs; after 60 minutes of dark adaptation, ERG amplitudes had recovered. Following 20 minutes of dark adaptation after indirect ophthalmoscopy (moderate-intensity light), significantly lower b-wave amplitudes were recorded in response to 2 of the brighter flash stimuli, compared with pretest ERGs; after 60 minutes of dark adaptation, ERG amplitudes had recovered. Following 20 minutes of dark adaptation after indirect ophthalmoscopy (bright-intensity light), all ERG amplitudes were significantly decreased and implicit times were significantly decreased at several flash intensities, compared with pretest ERGs; after 60 minutes of dark adaptation, ERG amplitudes and implicit times had returned to initial values, except for b-wave amplitudes recorded in response to dimmer stimuli.

Conclusions and Clinical Relevance—Results suggest that at least 60 minutes of dark adaptation should be allowed before ERGs are performed in dogs after fundus photography or indirect ophthalmoscopy. (*Am J Vet Res* 2005;66:1798–1804)

Electroretinography (ERG) is used in the assessment of retinal function. A dark-adapted (scotopic) intensity ERG series is a commonly used protocol that provides information about the rod photoreceptor response threshold, and the data collected can be analyzed to give a measure of retinal sensitivity.[1] During low-intensity flash stimulation, an ERG records the response from rod photoreceptors predominantly, whereas at brighter light intensities, it records a mixed rod-cone photoreceptor response. Electroretinography can be used for the early detection of photoreceptor dysfunction or loss, such as that resulting from the group of conditions known as **progressive retinal atrophies (PRAs)** and other retinopathies. Electroretinographically detectable changes develop prior to ophthalmoscopically detectable fundic changes in many forms of PRA and can be used for the early identification of such conditions. In dogs with suspected retinal disease, comprehensive examinations of the fundus, often including fundus photography, may be performed prior to making the decision to obtain a detailed ERG. After exposure of an animal to bright examination lights, a longer period of dark adaptation may be required to achieve full rod functional recovery, compared with the period of dark adaptation required by an animal that has been exposed to ambient room lighting. The **International Society for Clinical Electrophysiology of Vision (ISCEV)** standards for clinical ERG in humans recommend that fluorescein angiography or fundus photography is avoided prior to ERG; if those procedures are performed, then the dark-adaptation time should be increased from 20 minutes to 1 hour.[2] The ERG committee of the **European College of Veterinary Ophthalmologists (ECVO)** suggested a standard protocol for clinical ERG in dogs that is similar to the ISCEV standard for humans. The ECVO standards recommend that dark-adaptation time be increased from 20 minutes to 60 minutes in dogs if fundus photography or fluorescein angiography is performed prior to the ERG examination.[3] However, to the authors' knowledge, there are no published studies to verify that increasing the dark-adaptation time to 60 minutes in such circumstances is sufficient. Also, although it has been reported in humans that indirect ophthalmoscopic examination prior to ERG evaluation increases rod photoreceptor thresholds,[4] there are no published

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recommendations for duration of dark-adaptation time in dogs that undergo indirect ophthalmoscopic examination prior to ERG testing.

The purpose of the study reported here was to investigate the duration of dark-adaptation time required for recovery of ERG responses in dogs that have undergone either fundus photography or indirect ophthalmoscopy. Our intention was to investigate whether a 60-minute period of dark adaptation after either procedure was adequate to allow recovery of rod photoreceptor function to a level comparable with that determined after exposure of dogs to ambient room lighting prior to the 20-minute dark-adaptation period. We elected to use a scotopic-intensity series of ERGs, rather than follow the protocol suggested by the ECVO ERG committee, to make a more detailed assessment of rod function.

Materials and Methods

Animals—Six healthy crossbred dogs (age range, 6 months to 2 years) were used in the study. During the period of the study, the dogs' housing conditions included a daily cycle of 12 hours of light and 12 hours of dark, and the dogs were monitored for the presence or development of ocular abnormalities. Each of 6 experimental protocols was performed on each of the 6 dogs with a period of at least 1 week between subsequent procedures. The experiments were approved by the Institutional Animal Use and Care Committee of the University of Michigan and were in accordance with the humane principles set forth in the Public Health Service Policy on Humane Care and Use of Laboratory Animals (Health Research Extension Act of 1985) and the Association for Research in Vision and Ophthalmology resolution on ocular experimentation.

Preparation for ERG—Electroretinography was performed in anesthetized dogs. The dogs were premedicated with acepromazine maleate (0.05 mg/kg, IM). Anesthesia was induced by use of thiopental sodium (6 to 8 mg/kg, IV) and maintained via inhalation of isoflurane (2.5% delivered in oxygen). Body temperature was maintained with a heating pad. Depth of anesthesia was kept constant during the procedure, and oxygen saturation was monitored via pulse oximetry.

Maximal pupillary dilation in the left eye of each dog was achieved by applying 1% tropicamide and 10% phenylephrine hydrochloride. Pupillary dilation was measured before and after each ERG to ensure that maximal pupillary dilation was maintained. The dogs were positioned in right lateral recumbency, and the right eye was taped closed. The ERGs were recorded from the left eye, which was positioned in primary gaze by use of conjunctival stay sutures. A contact lens ERG recording electrode[a] was placed on the cornea with an application of 2.5% hydroxypropyl methylcellulose. A reference needle electrode was placed SC over the left zygomatic arch (5 cm caudal to the lateral canthus). A ground electrode was placed SC over the dorsal aspect of the neck.

ERG procedures—A scotopic-intensity series of ERGs was recorded from the left eye of each dog by use of an ERG system.[b] This instrument was selected because it is commonly used by veterinary ophthalmologists in the United States. The light-emitting diode unit supplied with the ERG system delivers white flashes of light. It was positioned 7 cm from the cornea along the visual axis of the eye to ensure consistent stimulation throughout the experiment. The band pass was set at 0.3 to 500 Hz and responses averaged. After 20 minutes of dark adaptation, a series of 8 white flashes (intensity range, –2.6 to 0.8 log **candela-seconds [cd-s]**/m^2) was delivered to the eye and the intensity series of ERGs were recorded as a pretest control. The time between flashes was increased from 1 second for the dim flashes to 50 seconds for the brighter flashes to avoid light adaptation of the rod photoreceptors. For the low flash intensities of –2.6 and –2.2 log cd-s/m^2, 32 flashes were averaged; for flash intensities of –1.6 and –1.0 log cd-s/m^2, 16 flashes were averaged; for flash intensities of –0.6 and 0.0 log cd-s/m^2, 8 flashes were averaged; for flash intensity of 0.4 log cd-s/m^2, 4 flashes were averaged; and for the brightest flashes at 0.8 log cd-s/m^2, 2 flashes were averaged.

Effect of fundus photography on ERG responses—To evaluate the influence of fundus photography on ERGs, a handheld fundus camera[c] was used. The camera was set at a flash intensity of 2 and viewing intensity of 4. Photometry performed by use of a photometer[d] indicated that the focus-

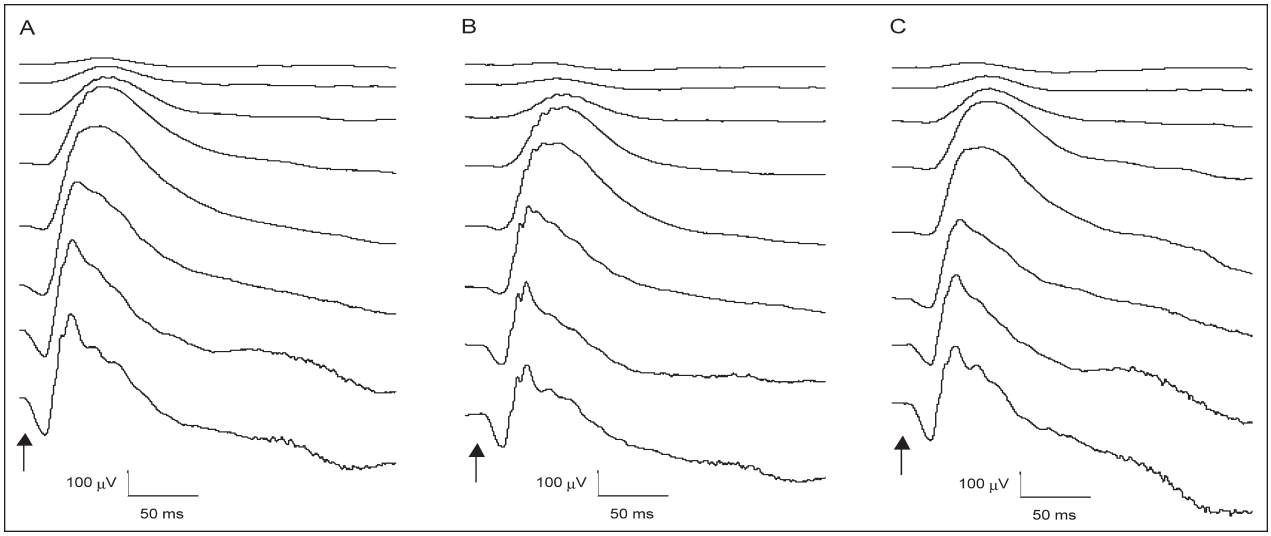

Figure 1—Representative electroretinographic (ERG) responses (intensity series) recorded from the left eye of an anesthetized dog prior to (A; pretest control) and following 20 (B) and 60 (C) minutes of dark adaptation after fundus photography (10 photographs obtained [1 every 30 seconds]). In each panel, arrow indicates the onset of flash stimuli; stimulus intensities (from top to bottom) are –2.6, –2.2, –1.6, –1.0, –0.6, 0.0, 0.4, and 0.8 log candela-seconds [cd-s]/m^2. Vertical bars = Amplitude (100 μV). Horizontal bars = Time (50 milliseconds).

ing background luminance was 12 cd/m^2 and flash intensity was 62.5 cd-s/m^2. Following pretest control ERG, 10 photographs of the left fundus were obtained (1 every 30 seconds). Then the intensity series of ERGs was repeated following either 20 or 60 minutes of dark adaptation.

Effect of indirect ophthalmoscopy on ERG responses—Indirect ophthalmoscopic examinations were performed at either a moderate or bright light intensity. For the moderate-intensity examinations, 1 model of indirect ophthalmoscope[e] was used with the intensity set as needed to examine the tapetal fundus (luminance measured at 117 cd/m^2). For the bright-intensity examinations, another model of indirect ophthalmoscope[f] was used with the intensity set as needed to examine a pigmented nontapetal fundus (luminance measured at 1,693 cd/m^2). The fundus examination involved moving the light over all quadrants to simulate a typical ophthalmoscopic examination. Following pretest control ERG, indirect ophthalmoscopy by use of a panretinal lens[g] was performed for 5 minutes, followed by an intensity series of ERGs recorded after either 20 or 60 minutes of dark adaptation.

Data analyses—Electroretinographic parameters (a- and b-wave amplitudes and implicit times) were measured. Amplitudes were measured in microvolts and included the a-wave amplitude from the onset of flash stimulus to the trough of the first negative potential and the b-wave amplitude from a-wave trough to peak of the b-wave (not including the effect on the b-wave of oscillatory potentials). Implicit times were measured in milliseconds and included the a-wave implicit time (time from onset of flash stimulus to the a-wave trough) and the b-wave implicit time (time from onset of flash stimulus to time of peak b-wave response). The ERG amplitudes and implicit times were analyzed by use of a Kolmogorov-Smirnov goodness-of-fit test to assess whether the data were normally distributed (a value of $P > 0.05$ was used to indicate a normal distribution).[h] As the data were

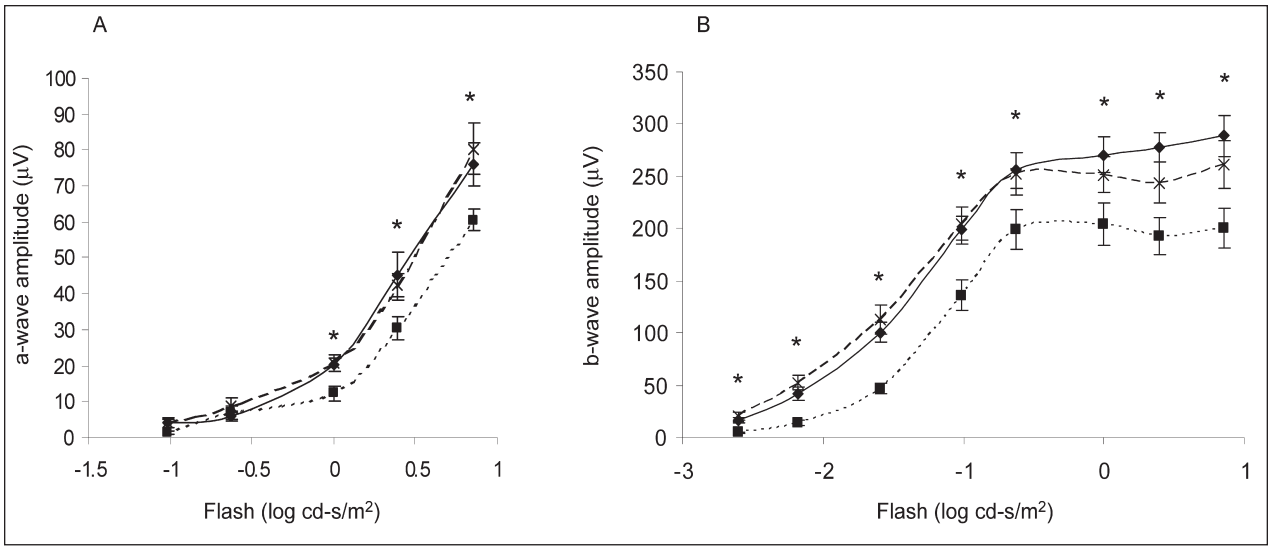

Figure 2—Mean ± SEM a-wave (A) and b-wave (B) ERG amplitudes recorded from the left eyes of 6 anesthetized dogs prior to (pretest control; diamonds) and following 20 (squares) and 60 (crosses) minutes of dark adaptation after fundus photography (10 photographs obtained [1 every 30 seconds]) plotted against stimulus intensities. *Mean ERG amplitude after 20 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value.

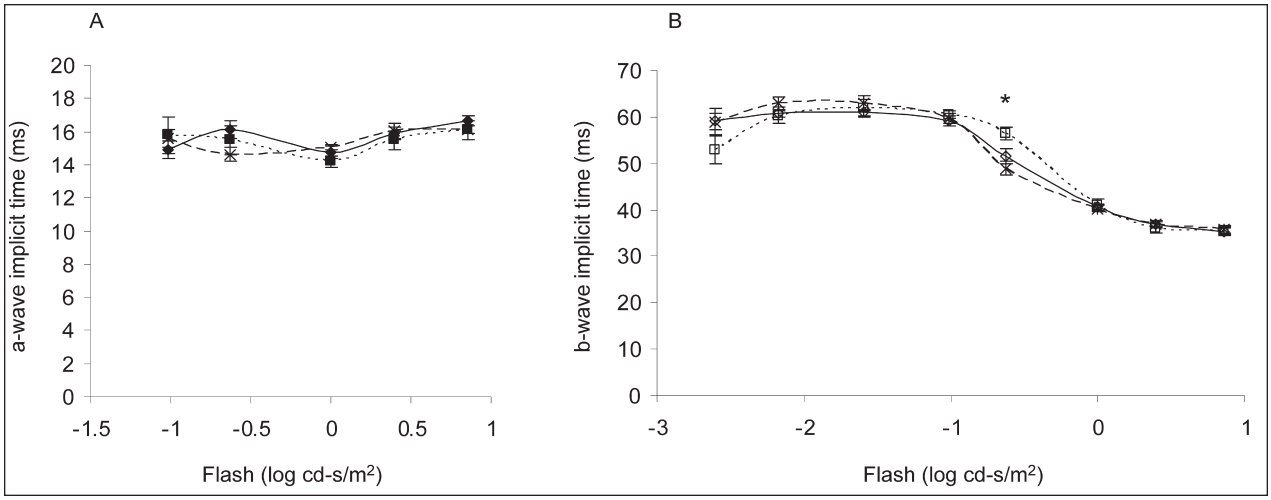

Figure 3—Mean ± SEM a-wave (A) and b-wave (B) implicit times recorded from the left eyes of 6 anesthetized dogs prior to (pretest control; diamonds) and following 20 (squares) and 60 (crosses) minutes of dark adaptation after fundus photography (10 photographs obtained [1 every 30 seconds]) plotted against stimulus intensities. *Mean implicit time after 20 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value.

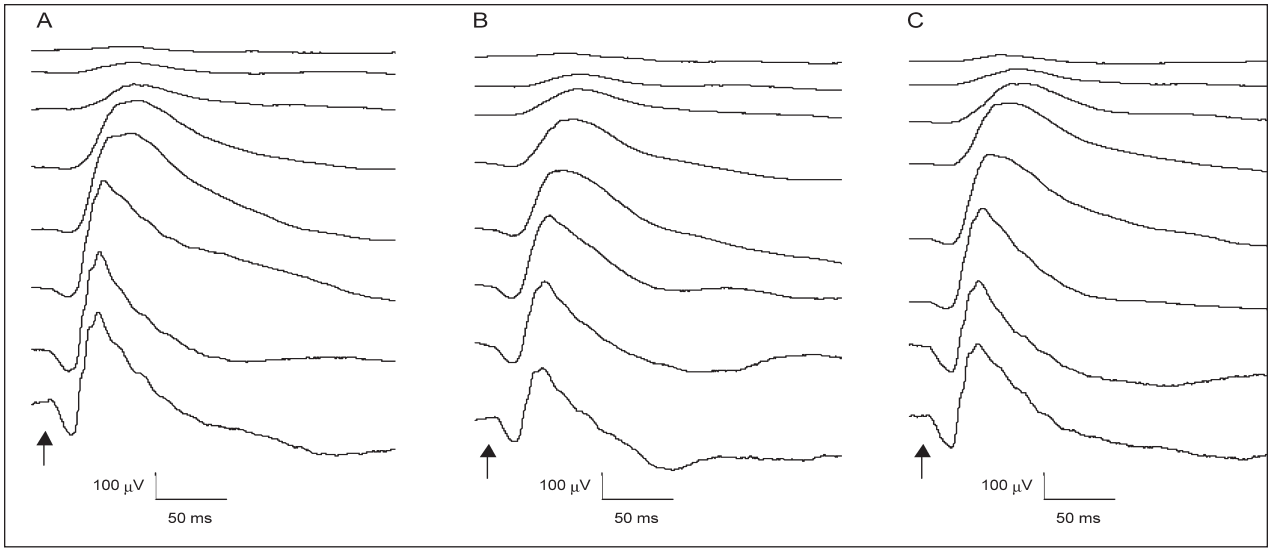

Figure 4—Representative ERG responses (intensity series) recorded from the left eye of an anesthetized dog prior to (A; pretest control) and following 20 (B) and 60 (C) minutes of dark adaptation after indirect ophthalmoscopy (performed with moderate-intensity light). In each panel, arrow indicates the onset of flash stimuli; stimulus intensities (from top to bottom) are –2.6, –2.2, –1.6, –1.0, –0.6, 0.0, 0.4, and 0.8 log cd-s/m^2. Vertical bars = Amplitude (100 μV). Horizontal bars = Time (50 milliseconds).

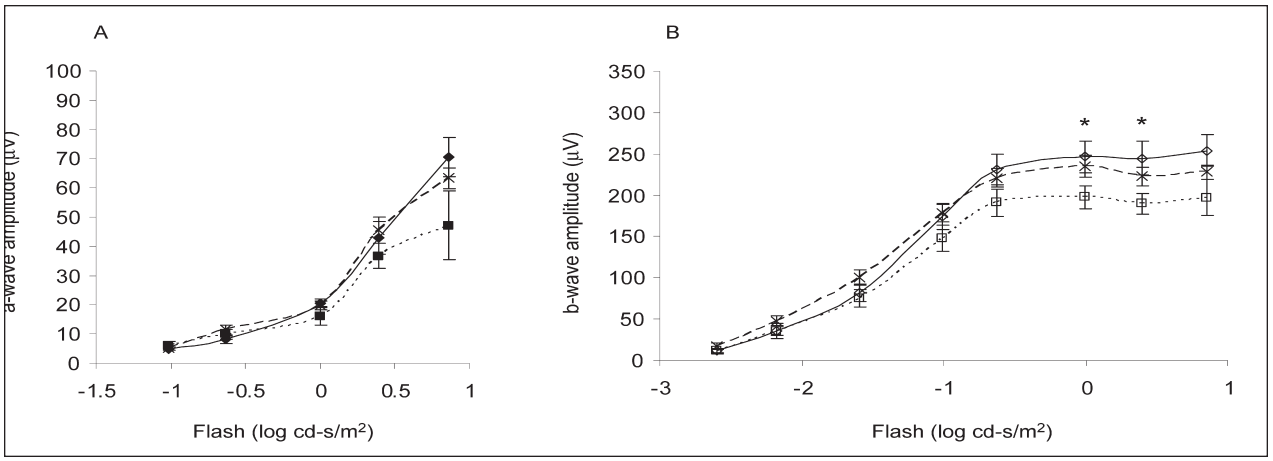

Figure 5—Mean ± SEM a-wave (A) and b-wave (B) ERG amplitudes recorded from the left eyes of 6 anesthetized dogs prior to (pretest control; diamonds) and following 20 (squares) and 60 (crosses) minutes of dark adaptation after indirect ophthalmoscopy (performed with moderate-intensity light) plotted against stimulus intensities. *Mean ERG amplitude after 20 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value.

found to be normally distributed, mean pretest control ERG values and mean posttest ERG values were compared and analyzed by use of a repeated-measures ANOVA and a Fisher protected least significant difference post hoc test. Values of $P < 0.05$ were considered significant.[h]

Results

At the initial evaluation, the dogs used in the study were free of ocular abnormalities; during the study period, ocular abnormalities did not develop in any dog. A comparison of all the pretest ERGs recorded from each dog did not reveal any significant differences in amplitudes and implicit times among the individual animals (data not shown).

Recovery of ERG responses (scotopic-intensity series) after fundus photography—Compared with the pretest control ERG findings, the ERG tracings recorded following fundus photography and 20 minutes of dark adaptation had lower amplitudes (**Figure 1**). The mean a-wave amplitudes were significantly lower than the pretest control amplitudes for the brighter flash stimuli, and mean b-wave amplitudes were significantly lower for all flash stimuli (**Figure 2**). Following 60 minutes of dark adaptation after fundus photography, the mean a-wave amplitudes had returned to the pretest control values. The mean b-wave amplitudes were still slightly decreased at bright intensities, compared with the pretest control values, but this difference was not significant. Mean a- and b-wave implicit times were not different before or after fundus photography except for a significant increase in mean b-wave implicit time for 1 stimulus intensity (–0.6 log cd-s/m^2) determined after 20 minutes of dark adaptation following fundus photography (**Figure 3**).

Recovery of ERG responses (scotopic-intensity series) after indirect ophthalmoscopic examination

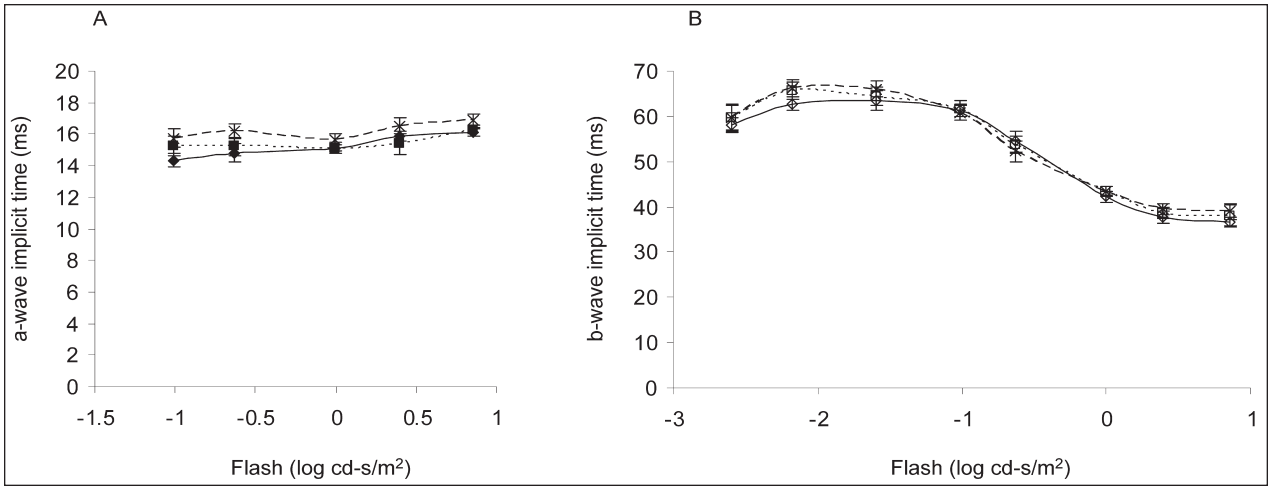

Figure 6—Mean ± SEM a-wave (A) and b-wave (B) implicit times recorded from the left eyes of 6 anesthetized dogs prior to (pretest control; diamonds) and following 20 (squares) and 60 (crosses) minutes of dark adaptation after indirect ophthalmoscopy (performed with moderate-intensity light) plotted against stimulus intensities. After 20 or 60 minutes of dark adaptation, none of the mean a- or b-wave implicit times were significantly different from the pretest control values.

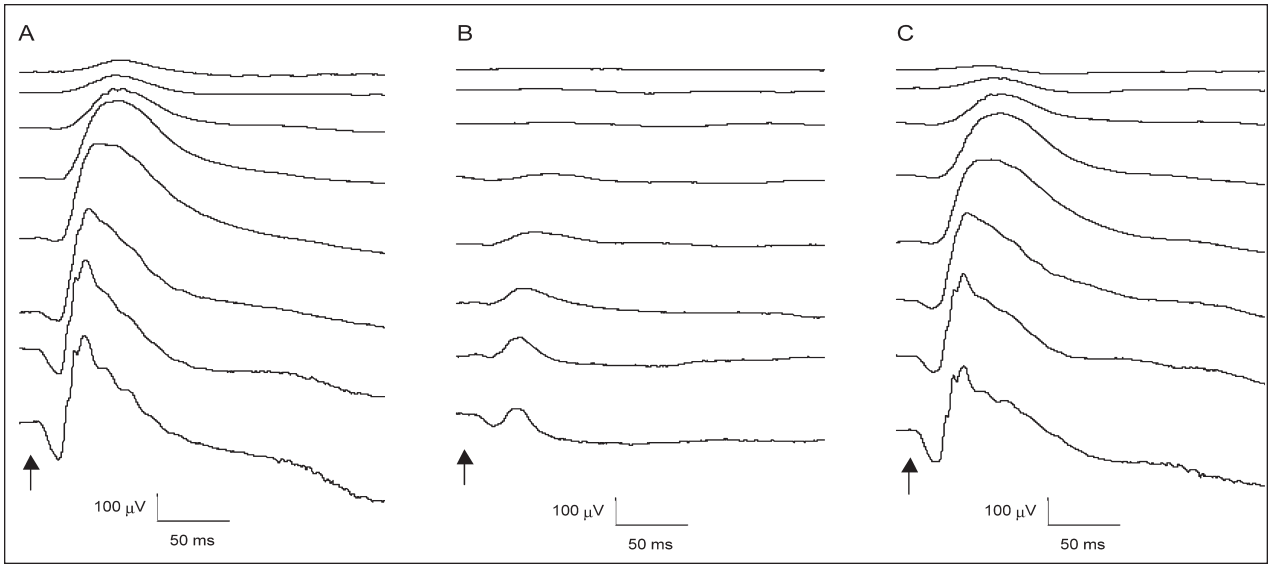

Figure 7—Representative ERG responses (intensity series) recorded from the left eye of an anesthetized dog prior to (A; pretest control) and following 20 (B) and 60 (C) minutes of dark adaptation after indirect ophthalmoscopy (performed with moderate-intensity light). In each panel, arrow indicates the onset of flash stimuli; stimulus intensities (from top to bottom) are –2.6, –2.2, –1.6, –1.0, –0.6, 0.0, 0.4, and 0.8 log cd-s/m^2. Vertical bars = Amplitude (100 μV). Horizontal bars = Time (50 milliseconds).

involving moderate-intensity light—After 20 or 60 minutes of dark adaptation following indirect ophthalmoscopic examination performed by use of a moderate-intensity light, there were no significant differences in mean a-wave amplitudes, compared with the pretest control values. After 20 minutes of dark adaptation, mean b-wave values were lower than pretest values, but this difference was significant for only 2 of the higher flash intensities used (0.3 and 0.4 log cd-s/m^2; **Figures 4 and 5**). After 60 minutes of dark adaptation, mean ERG amplitudes were not significantly different from the pretest control values. The mean implicit times for a- and b-waves after 20 or 60 minutes of dark adaptation following indirect ophthalmoscopic examination performed by use of a moderate-intensity light were not different from the respective pretest control values (**Figure 6**).

Recovery of ERG responses (scotopic-intensity series) after indirect ophthalmoscopic examination involving bright-intensity light—After 20 minutes of dark adaptation following indirect ophthalmoscopic examination involving a bright-intensity light, the amplitudes of the ERG tracings were notably decreased and had highly decreased oscillatory potentials, compared with the pretest control values (**Figure 7**). The b-wave response threshold was approximately 1.5 log-units higher than that of the control ERG series, and both mean a- and b-wave amplitudes were significantly decreased for all light intensities (**Figure 8**). After 60 minutes of dark adaptation, mean a-wave amplitudes were not significantly different from the pretest control values; the mean b-wave amplitudes had not returned to the pretest values, but these differences were only significant at lower flash intensities (–1.0 log cd-s/m^2

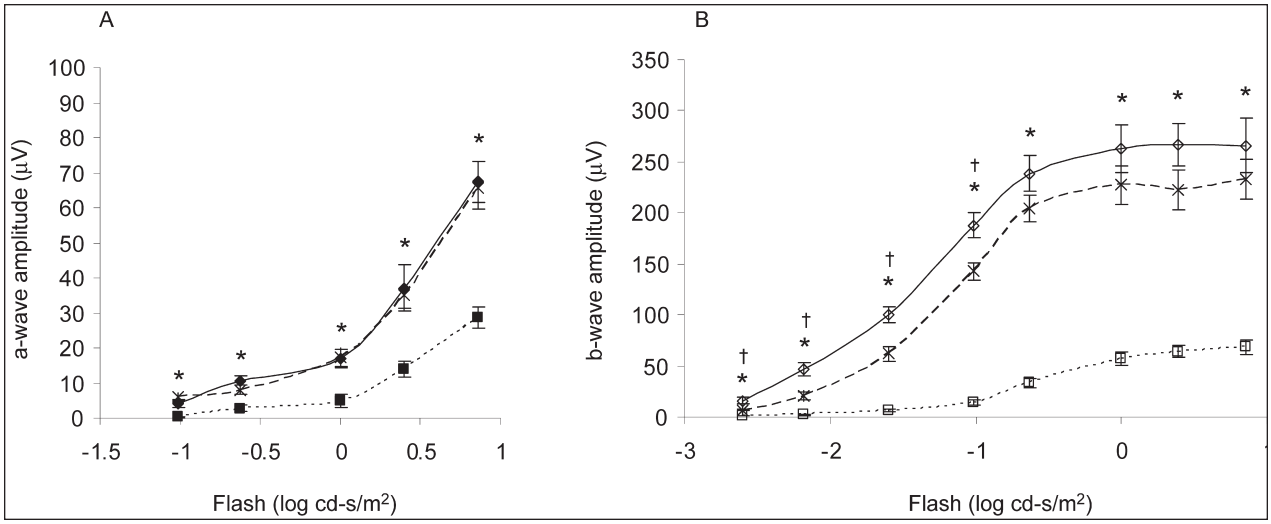

Figure 8—Mean ± SEM a-wave (A) and b-wave (B) ERG amplitudes recorded from the left eyes of 6 anesthetized dogs prior to (pretest control; diamonds) and following 20 (squares) and 60 (crosses) minutes of dark adaptation after indirect ophthalmoscopy (performed with bright-intensity light) plotted against stimulus intensities. *Mean ERG amplitude after 20 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value. †Mean ERG amplitude after 60 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value.

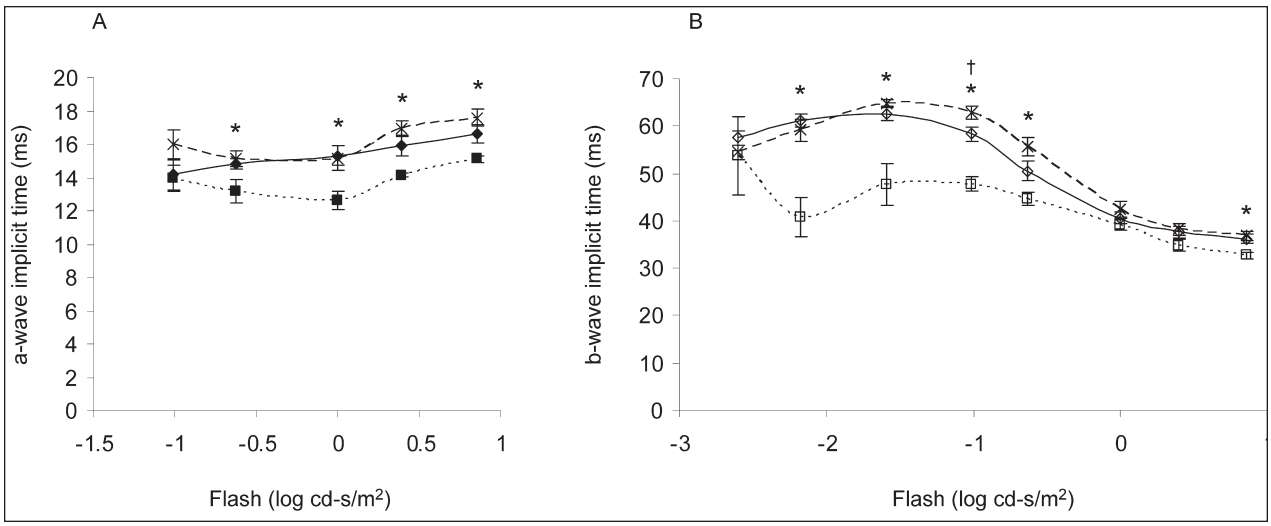

Figure 9—Mean ± SEM a-wave (A) and b-wave (B) implicit times recorded from the left eyes of 6 anesthetized dogs prior to (pretest control; diamonds) and following 20 (squares) and 60 (crosses) minutes of dark adaptation after indirect ophthalmoscopy (performed with bright-intensity light) plotted against stimulus intensities. *Mean implicit time after 20 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value. †Mean implicit time after 60 minutes of dark adaptation is significantly ($P < 0.05$) different from pretest control value.

and lower). Following 20 minutes of dark adaptation, mean a-wave implicit times for all stimulus intensities except the intensity closest to a-wave threshold (ie, –1.0 log cd-s/m^2) were significantly decreased, as were mean b-wave implicit times for 5 of the 8 stimulus intensities (–2.2, –1.6, –1.0, –0.6, and 0.8 log cd-s/m^2), compared with the pretest control values (**Figure 9**). After 60 minutes of dark adaptation, mean b-wave implicit times were not significantly different from pretest control values, except for 1 stimulus intensity (–1.0 log cd-s/m^2) at which the mean b-wave implicit time was significantly increased.

Discussion

Decreases in scotopic ERG amplitudes and altered ERG thresholds can be indicative of the early stages of progressive hereditary retinal degenerative disorders such as the PRAs.[5–7] The early detection of such disorders is valuable for dog breeders, particularly in breeds for which DNA-based tests for causal gene mutations have not been developed. Several factors can lead to differences in apparent ERG threshold, amplitudes, and implicit times. There are variations intrinsic to the individual being tested, differences imposed by the recording equipment, and physiologic differences imposed by the method of ERG used. The degree of dark adaptation is one of these variables. If the individual is not fully dark adapted, ERG responses generated from the rod photoreceptor pathways will not be maximal. This could lead to a misdiagnosis of retinal dysfunction. After exposure of a dog to a bright-light stimulus, a longer period of dark adaptation is required

to allow full recovery of rod photoreceptor-generated responses than that required if the dog had previously been exposed to lower intensities of light.[8] The lack of published studies to indicate whether the recommendations made by the ECVO ERG committee[3] for the duration of dark adaptation following fundus photography are adequate to allow full recovery of ERG responses makes it an act of faith to assume that the period of dark adaptation recommended by ISCEV[2] for humans will be applicable to dogs.

Results of the present study in dogs indicate that after fundus photography (performed as the sole procedure during which 10 photographs are obtained [1 every 30 seconds]), 60 minutes of dark adaptation is sufficient. After procedures in which more fundus photographs are obtained, a brighter flash or illuminating light is used, or the photographs are obtained over a longer period (thus increasing a dog's exposure to the examination light), it is possible that a longer period of dark adaptation would be required.

The published recommendations[3] of the ECVO ERG committee do not mention any need to increase the period of dark-adaptation time if indirect ophthalmoscopy was performed prior to ERG evaluation in dogs. In the present study, we investigated whether it was necessary to increase dark-adaptation time after indirect ophthalmoscopy involving 2 intensities of examination light. The 2 intensities were chosen to represent the intensities of light typically required to examine either pigmented nontapetal fundi or more reflective tapetal fundi. Photoreceptors in the tapetal area of the retina are likely to receive greater light stimulation from a light of a given intensity than photoreceptors in the nontapetal area because the tapetum reflects the light back through the retina rather than absorbing the light energy (as occurs in the darkly pigmented areas of the fundus). The results of the present study indicated that 60 minutes of dark adaptation was sufficient for recovery of ERG responses after an indirect ophthalmoscopic examination of 5 minutes' duration with a light intensity suitable for examination of the tapetal fundus in dogs. When a brighter ophthalmoscope setting was applied for a 5-minute indirect ophthalmoscopic examination of each dog, mean ERG b-wave amplitudes were still significantly decreased (compared with pretest control values) in response to the 4 dimmest flash stimuli in the intensity series after a 60-minute period of dark adaptation. An indirect ophthalmoscopic examination of longer duration would be expected to have resulted in an even slower recovery of ERG responses. Indirect ophthalmoscopic examinations of more than 5 minutes' duration might be used when unusual lesions are being examined or in situations where multiple examiners are involved, such as at a teaching institution. Under such circumstances, the prolonged examination may include fundus photography. Our data suggest that 60 minutes of dark adaptation is sufficient for recovery of ERG responses in dogs that have undergone an indirect ophthalmoscopic examination (involving a moderate-intensity light) of 5 minutes' duration or less. Indirect ophthalmoscopy performed with a brighter examination light and possibly the combination of indirect ophthalmoscopy with fundus photography might cause suppression of rod photoreceptor responses for a longer period; in such circumstances, a longer period of dark adaptation would most likely be required. Minimization of retinal exposure to bright light stimuli prior to dark adaptation for ERG recording is recommended. If it is essential that dogs undergo photography or indirect ophthalmoscopy prior to an ERG evaluation, the light intensity should be kept low, the duration of light exposure should be minimized, and a period of dark adaptation of at least 60 minutes' duration should be allowed before proceeding with ERG.

a. ERG-jet, Universo Plastique, Grenchen, Switzerland.
b. BMP-100 electroretinographic system, version 5.50, RetinoGraphics Inc, Norwalk, Conn.
c. RC-2 fundus camera, Kowa Ltd, Tokyo, Japan.
d. Research radiometer IL 1700 with SED033 silicon light detector, International Light Inc, Newburyport, Mass.
e. Heine Omega 180, Heine Instruments, Herrshing, Germany.
f. Keeler Dualite KM1, Keeler Inc, Windsor, England.
g. Volk 2.2 panretinal lens, Volk Optical Inc, Mentor, Ohio.
h. SAS, version 9.1, SAS Institute Inc, Cary, NC.

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