Effects of chemical restraint on electroretinograms recorded sequentially in awake, sedated, and anesthetized dogs

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Objective—To quantitatively and qualitatively compare electroretinography (ERG) recordings in awake, sedated, and anesthetized dogs.

Animals—Six 6-month-old Beagles.

 Procedures—A brief ERG protocol for dogs was used. Following 1-minute and subsequent 5-minute dark adaptation, mixed rod-cone responses were recorded bilaterally with a handheld multispecies ERG device with dogs in each of 3 states of consciousness: awake, sedated (dexmedetomidine and butorphanol), and anesthetized (atropine and hydromorphone, followed by propofol and midazolam and anesthetic maintenance with isoflurane). Low- and high-frequency noise levels were quantified via Fourier analysis, and the effect of consciousness state on signal amplitude, implicit time, and noise was analyzed via repeated-measures ANOVA. In addition, 13 veterinary ophthalmologists who were unaware of the dogs’ consciousness states subjectively graded the ERG recording quality, and scores for each tracing were compared.

Results—ERG amplitudes were highest in awake dogs and lowest in anesthetized dogs. Implicit times were shortest in awake dogs and longest in anesthetized dogs. Differences in b-wave amplitudes and a-wave implicit times were significant. Neither low- nor high-frequency noise levels differed significantly among consciousness states. Furthermore, no significant differences were identified among observers’ scores assigned to ERG tracings.

Conclusions and Clinical Relevance—Anesthesia and sedation resulted in significant attenuation and delay of ERG responses in dogs. Chemical restraint of dogs had no consistently significant effect on low- or high-frequency noise levels or on observer perception of signal quality. (Am J Vet Res 2013;74:1036–1042)

Electroretinography, which involves recording of an electrical response generated by retinal neurons and supporting cells when the eye is stimulated by light, is performed routinely in veterinary ophthalmology. The light stimulation creates ionic changes within the intra- and extracellular spaces, which can be measured as electrical potentials\textsuperscript{1,2} that assume a predictable waveform. The initial negative deflection of the waveform is basically an indicator of photoreceptor hyperpolarization and is referred
longer protocols considered more diagnostic for rod and cone disease. The 2 most common indications for ERG in a veterinary ophthalmology practice are to assess retinal function prior to cataract surgery and to distinguish among distal optic neuritis, central blindness, and sudden acquired retinal degeneration syndrome in patients with amaurosis with a clinically normal fundus. In both of these instances, the clinician is simply looking for a brief yes-or-no response to indicate the prognosis for vision following cataract surgery or to rule out sudden acquired retinal degeneration syndrome. In such situations, a brief protocol with a mean duration of approximately 10 min/eye, including dark adaptation, is used to assess overall retinal function.

Published standard ERG protocols suggest that all animals must be anesthetized, particularly for long ERG sessions typically used in the diagnosis of inherited photoreceptor diseases such as progressive rod-cone degeneration or achromatopsia.1,3 The reasons for this recommendation are to minimize noise and artifacts associated with blinking or other movement, to facilitate patient handling, and to maintain the electrodes in their proper position. Despite these recommendations, many veterinary ophthalmologists perform ERG in awake or sedated dogs.

The reluctance of some clinicians to perform ERG on an anesthetized patient when the yes-or-no method is to be used is likely because of the high financial cost to the client, possible risk to the patient, and increase in time required for the procedure, compared with in nonanesthetized subjects. Although some clinicians may consider sedation an acceptable alternative to anesthesia for ERG, some of the same financial, time, and health concerns can remain. Additionally, use of sedatives and anesthesia alters the ERG a- and b-wave amplitudes and implicit times and these effects must be taken into consideration when evaluating the ERG response.

The purpose of the study reported here was to quantitatively and qualitatively compare ERG recordings obtained in awake, sedated, and anesthetized dogs. Our hypothesis was that use of anesthesia, and to a lesser extent sedation, would reduce low- and high-frequency noise associated with the ERG recording, compared with noise in ERG performed in awake dogs, thereby confirming the benefits of performing ERG in anesthetized dogs. We also expected that sedation and anesthesia would prolong the a- and b-wave implicit times and decrease the a- and b-wave amplitudes.

Materials and Methods

Animals—Six 6-month-old female laboratory Beagles were used for the study. All dogs received general physical and ophthalmic examinations by the same investigator (KSF) immediately prior to the study. The ophthalmic examination was performed by means of slit-lamp biomicroscopy,8 indirect ophthalmoscopy,6 and rebound tonometry.8 The study protocol was approved by the Animal Care and Use Committee of the University of California-Davis.

Experimental protocol—Electroretinograms of both eyes were recorded in each dog at 3 consciousness states: awake, sedated, and anesthetized. Recording in the awake state followed by recording in the sedated state were performed on the same day, with approximately 30 minutes separating the recordings. The eye order was chosen in an inconsistent manner and varied with dog and consciousness state.

After the awake recording was made, the hydroxypropyl methylcellulose8 applied for the ERG recording was irrigated from the eyes and a 22-gauge IV catheter was placed in a cephalic vein. Each dog was then sedated by IV administration of dexmedetomidine hydrochloride8 (2.5 µg/kg) and butorphanol8 (0.1 mg/kg). After 5 minutes had elapsed, dogs were positioned in lateral recumbency. Heart rate was measured with a stethoscope,8 and blood pressure measurements were obtained with an oscillometric devise,8 with the cuff placed on the right hind limb. After the ERG recordings were completed in both eyes, atipamezole8 (volume equal to that of dexmedetomidine) was administered IM to reverse the effects of the dexmedetomidine. Heart rate and blood pressure were monitored until both variables returned to presedation values.

Between 48 and 72 hours later, hydromorphone hydrochloride1 (0.05 mg/kg) and atropine sulfate1 (0.02 mg/kg) were administered to each dog and a 22-gauge IV catheter was placed in a cephalic vein. Anesthetic induction was achieved with propofol1 (4 mg/kg) and midazolam1 (0.1 mg/kg) administered IV to effect, and anesthesia was maintained with 1.2% isoflurane1 in oxygen. Each dog was placed on a ventilator and given atracurium8 (0.1 mg/kg, IV). Monitoring was performed through oscilometric blood pressure readings, capnography, pulse oximeter, paralysis assessment with a train-of-four test, and cardiac auscultation. Anesthesia was maintained for 15 minutes to ensure a stable depth of anesthesia had been obtained before ERG began. Dogs were allowed to recover from anesthesia as soon as paralysis had dissipated.

ERG—A handheld multispecies ERG device9 was used, which was equipped with a mini Ganzfeld for complete retinal stimulation with limited light scattering.9 For each pair of ERG recordings, pupillary dilation of both eyes was first achieved in each dog with tropicamide,9 the eyelids were gently retracted manually, and proparacaine hydrochloride9 and 2.5% hydroxypropyl methylcellulose9 were applied to each eye. A monopolar electrode contact lens was used as the active electrode.9 Two subdermal needle electrodes were placed 2 cm from the lateral canthus and in the pinna and served as reference and ground electrodes, respectively.9 All electrode placement was conducted in ambient room light.

A preprogrammed short ERG protocol was used, which is a quick protocol that provides for a brief yes-or-no mixed rod and cone response in a scotopic environment. This is a standard protocol included in the database of the handheld multispecies electroretinograph device. Responses to a standard flash (3 cd·s/m2; mean, 4 flashes; 0.1 Hz) were recorded after both 1 and 5 minutes of dark adaptation.

Calculations—Determination of a- and b-wave amplitudes and implicit times was performed by identifying the respective trough and peak. To quantify noise
levels, recordings were exported to an FFT graph. The FFT breaks down the signal into its constituent fundamental frequency components or harmonics. When the handheld multispecies ERG device is used, the breakdown results in 80 harmonics at a resolution of 5 Hz (0 to 400 Hz). The first 5 harmonics (0 to 25 Hz), which contain most of the biological activity, were summed and defined as signal. The sum of the next 5 harmonics (26 to 50 Hz) was defined as low-frequency noise, whereas the sum of the last 5 harmonics (376 to 400 Hz) was defined as high-frequency noise. Signal-to-low-frequency noise ratio and signal-to-high-frequency noise ratio were calculated by dividing the signal by the noise.

Questionnaire—Thirteen veterinarians, 7 of whom were in veterinary ophthalmology training programs (residents) and 6 of whom were board-certified ophthalmologists or had PhDs and were highly experienced in ERG (experts), replied to a questionnaire. The respondents were not informed about the study objectives or methods and were therefore unaware of the consciousness state of the dog evaluated or eye represented by a tracing. The numbers assigned to the ERG tracings in the questionnaires were randomly generated and assigned to each ERG recording.

In the questionnaire, respondents were asked to grade the recording quality of the 6 sets of recordings from each dog. Each set included 2 tracings (1 and 5 minutes) for each dog, each eye, and each consciousness state. For example, a dog had 2 tracings for the left eye in the awake state (equaling 1 set), 2 tracings for the left eye in the sedated state, 2 tracings for the left eye in the anesthetized state, and a similar number of tracings for the right eye in each consciousness state. Respondents were asked to rank the quality of the sets on a scale of 1 (best) to 6 (worst) for each dog (ie, “Which set do you like the most and which set do you like the least?”), taking into account all aspects of the set, including amplitudes, noise levels, wave shape, and overall quality.

Statistical analysis—Within-factor repeated-measures ANOVA was performed with the aid of statistical software 3 times to simultaneously evaluate the effect of consciousness state (awake, sedated, or anesthetized) and control for eye (right vs left), time of reading (1 and 5 minutes), and all possible interactions on the dependent variables (amplitude, implicit time, and high- and low-frequency noise). Values of $P < 0.05$ were considered significant.

Differences in median ranking for each dog among all observers for each of the 6 combinations of consciousness state and eye were evaluated via an exact Friedman test and different statistical software. The distributions of rankings for each consciousness state and for each of 6 combinations of consciousness state and observer expertise (resident vs expert) were graphed.

Table 1—Mean ± SD values for ERG variables measured bilaterally in six 6-month-old Beagles in awake, sedated, and anesthetized states.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Awake</th>
<th>Sedated</th>
<th>Anesthetized</th>
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<tbody>
<tr>
<td>a-wave amplitude (µV)</td>
<td>59.60 ± 16.32*</td>
<td>33.99 ± 26.08</td>
<td>32.60 ± 12.07</td>
</tr>
<tr>
<td>a-wave implicit time (ms)</td>
<td>11.85 ± 0.32†</td>
<td>13.72 ± 1.02</td>
<td>13.49 ± 1.08</td>
</tr>
<tr>
<td>b-wave amplitude (µV)</td>
<td>272.50 ± 54.67†</td>
<td>191.26 ± 68.93</td>
<td>125.58 ± 40.05</td>
</tr>
<tr>
<td>b-wave implicit time (ms)</td>
<td>33.81 ± 1.65*</td>
<td>43.19 ± 8.38</td>
<td>37.81 ± 8.17</td>
</tr>
<tr>
<td>High-frequency noise (dB)</td>
<td>460.54 ± 15.40</td>
<td>478.42 ± 13.89</td>
<td>471.67 ± 21.78</td>
</tr>
<tr>
<td>Low-frequency noise (dB)</td>
<td>121.88 ± 8.93</td>
<td>133.08 ± 9.13</td>
<td>121.96 ± 19.85</td>
</tr>
<tr>
<td>Signal-to-high-frequency noise ratio</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Signal-to-low-frequency noise ratio</td>
<td>0.39 ± 0.04</td>
<td>0.44 ± 0.05</td>
<td>0.41 ± 0.03</td>
</tr>
</tbody>
</table>

*Indicated value is significantly ($P < 0.05$) different from that for sedated dogs. †Indicated value is significantly ($P < 0.05$) different from that for sedated or anesthetized dogs.
ically displayed in side-by-side boxplots. Median scores of residents and experts for each dog were contrasted via the Wilcoxon signed rank test for paired data. Values of $P < 0.05$ were considered significant.

Results

Animals—All 6 Beagles were sexually intact females, with a mean ± SD body weight of 9.3 ± 0.4 kg. One dog was found to have bilateral, incipient posterior polar subcapsular cataracts; all other dogs had unremarkable findings of physical and opthalmic examinations.

ERG—Results of ERG recorded bilaterally in the 6 dogs were summarized (Table 1; Figures 1–3). Because no significant difference was identified between any of the dependent variables and time of reading for each

![Figure 2](image)

*Figure 2—Representative FFT graphs for the ERG tracings shown in Figure 1. The sum of the first 5 bars represents the ERG signal, the sum of the next 5 bars represent the low-frequency noise, and the sum of the last 5 bars represent the high-frequency noise. The graph for the ERG tracing with substantial noise (right panel) has more low- and high-frequency noise than the graph for the ERG tracing with minimal noise (left panel).*

![Figure 3](image)

*Figure 3—Representative ERG tracings from a 6-month-old Beagle showing the amplitude, waveform, and timing when the dog was in awake (A), sedated (B), and anesthetized (C) states. All 3 tracings are fairly noise free. The amplitude was higher when the dog was awake than when it was sedated and was higher when the dog was sedated than when it was anesthetized (notice the difference in y-axis scale).*
As expected, a- and b-wave amplitudes were highest in awake dogs. Values for a- (P = 0.040) and b- (P = 0.035) waves were significantly higher when dogs were awake than when they were sedated. The b-wave amplitudes were significantly (P = 0.003) higher in awake versus anesthetized dogs, and the a-wave amplitudes were higher but not significantly (P = 0.061) in awake versus anesthetized dogs. The a-wave implicit times were significantly shorter in awake versus sedated (P = 0.008) and anesthetized (P = 0.013) dogs, and the b-wave implicit times were significantly (P = 0.035) shorter in awake versus sedated dogs. The b-wave implicit time was not significantly different between awake and anesthetized states. Amplitude and implicit times in the anesthetized versus sedated state did not differ significantly for a- or b-waves.

No significant differences were evident among consciousness states for low- (P = 0.33) or high- (P = 0.14) frequency noise. The high- and low-frequency noise values following FFT (higher value equates to a lower amount of noise) were summarized (Table 1). Although differences were not significant, lower noise values were obtained when dogs were in the sedated state than when they were anesthetized or awake.

Quality scores assigned by the 7 resident and 6 expert observers to the ERG tracings for each dog in the 3 consciousness states were graphically displayed (Figure 4). No significant (P = 0.69) difference was identified among the scores for any consciousness state when no differentiation was made between resident and expert observers. Comparison of scores between resident and expert observers also revealed no significant differences for the awake (P = 0.25), sedated (P = 1.0), or anesthetized (P = 0.88) states (Figure 5).

**Discussion**

The unexpected results of the present study indicated no significant difference in high- or low-frequency ERG noise levels on the basis of whether young laboratory Beagles were awake, sedated, or anesthetized at the time the recordings were made. Additionally, veterinary ophthalmology residents and veterinary electrophysiology experts were unable to distinguish differences in the quality of ERG tracings pertaining to 6 dogs in any of the 3 consciousness states. Therefore, for the brief yes-or-no ERG protocol used, anesthesia and sedation were not needed to obtain a high-quality tracing even in young, untrained dogs.

The benefit of obtaining a high-quality, low-noise tracing in an awake dog is that there is no need to adjust for the impacts of sedation or anesthesia on the ERG waveform. Additionally, avoiding sedation or anesthesia eliminates the potential risks to the subject associated with these procedures as well as decreases both the cost of ERG to the client and the time required to perform the recordings. However, should a longer ERG protocol (eg, for diagnosing inherited retinal diseases) be used, it would be difficult to maintain cooperation of the dog and restrain it for the duration of the recording. In such a situation, movement artifacts and signal noise would likely be substantial, requiring the dog to be sedated or anesthetized, despite the drawbacks of chemical restraint.

On the other hand, the expectation that sedation and anesthesia of the dogs would prolong the a- and b-wave implicit times and decrease the a- and b-wave amplitudes was largely correct and supported findings of prior research. The sedation protocol used in the study led to lower a- and b-wave amplitudes and longer implicit times than when ERG was performed in awake dogs. This was not unexpected given the findings of prolonged implicit time and a decrease in amplitude response when medetomidine hydrochloride alone is
administered. In our study, the isomer of this sedative, dexmedetomidine, was chosen because of the known effects of α₂-adrenoceptor agonists on ERG amplitude and implicit timing. Given the cardiovascular effects of an α₂-adrenoceptor agonist and the potential risks of the drug in some cataract surgery patients (eg, elderly animals or those with cardiovascular compromise), our goal was to lessen the required dose of dexmedetomidine by combining it with butorphanol.

The anesthesia protocol used yielded mixed results. As expected, ERG tracing amplitudes were lower when dogs were anesthetized versus awake. Although not significant, a lower amplitude was also evident when dogs were anesthetized versus sedated. With a larger sample size, it is possible that difference would have become significant. Implicit times in the anesthetized state were not as predictable; they were longer than those of the awake state for the a-wave (as expected), and no significant difference was identified when the b-waves of anesthetized and awake dogs were compared or when a- and b-waves of anesthetized and sedated dogs were compared. A larger number of dogs may have increased the power to detect a significant difference if one existed.

The anesthetics chosen were a combination of sedatives and inhalation anesthetics that are routinely used for anesthetizing dogs undergoing cataract surgery. This included premedication with an opioid (hydromorphone) and an anticholinergic (atropine), induction of anesthesia with propofol, and anesthetic maintenance with isoflurane. Other inhalation anesthetics, such as sevoflurane and halothane, have been shown to have depressant effects on ERG tracings, but propofol has been shown to increase the amplitude of the b-wave.

Eye positioning can be a problem when performing ERG in sedated and anesthetized dogs. With moderate and deep sedation, the eye may rotate ventromedially, particularly with higher doses of sedatives. The dose used in the present study was lower than in another study, with the aim being to minimize problems with eye rotation. Likely because of the lower dose used and the short sedation period, eye rotation was not a factor in the study dogs when they were sedated. In situations when higher doses are used for sedation and eye rotation occurs, stay sutures can be used to centralize the eye. Eye rotation is also a major concern with anesthesia. For this reason, all dogs were temporarily paralyzed with atracurium to attain adequate eye positioning and were subsequently maintained on a ventilator to control respiration. Systemic paralysis exposes the ERG subjects to additional hazards, requires additional monitoring and expense, and often prolongs the procedure.

One factor that might have affected the results of the present study was the use of a brief ERG protocol with a mean of 4 flashes at each time point. The signal averaging may have improved the quality of the signal in all consciousness states, but particularly in the awake dogs. Additionally, with this brief protocol, after the first ERG recording, there is a 5-minute dark adaptation wait period during which the subject must remain with electrodes in place for 5 minutes. Although the electrodes can be replaced if they fall out during this period, this is another time point in which challenges might occur with an awake subject, particularly when a longer protocol is used. We chose a brief yes-or-no protocol, which only required 1 and 5 minutes of dark adaptation because the most common use of ERG in veterinary ophthalmology is for quick determination of retinal function prior to cataract surgery or for diagnosis of sudden acquired retinal degeneration syndrome. Although the protocol used and the brief dark adaptation times were appropriate for such clinical purposes, more in-depth protocols with longer dark adaptation would be necessary for a more thorough assessment of retinal disease.

Another possible variable in the clinical application of ERG is subject disposition. The laboratory Beagles used in the present study were energetic and untrained, but they were not aggressive. We noticed more movement when dogs were awake than when they were sedated or anesthetized; however, the individuals restraining the awake dogs were able to thwart any attempts to remove electrodes. Some dogs were more active and required more restraint than others. In a clinic setting when dealing with a particularly difficult, fearful, or aggressive dog or when using a longer ERG protocol, sedation or anesthesia may be advisable to prevent injury to the restrainer or patient.

Results of the present study suggested that the brief yes-or-no ERG protocol used can successfully and accurately be performed in a clinical setting on awake dogs without considerable noise or waveform compromise in the ERG tracings. A quality waveform can be obtained with dogs sedated or anesthetized, but such chemical restraint when performing brief ERG will lead to changes in the ERG wave amplitude and timing and will lengthen the duration of the procedure.

References