REVIEW

Technical issues in electrodiagnostic recording

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Abstract

Electroretinogram (ERG) and visual-evoked potentials (VEPs) are used in veterinary ophthalmology to assess the functional integrity of the retina and the central visual pathway. The interpretation of altered electrophysiologic potentials in diseases can be of great diagnostic value, although it is important to be aware of technical factors and the limitations of these techniques which may result in over-interpretation and misinterpretation of the recordings, such that they are similar to those found in disease-related electrophysiologic changes. The recorded potentials represent the differences in voltage between the active and reference electrodes. The ground electrode serves as zero. A differential or instrumentation amplifier selectively amplifies signals of interest while rejecting noise. Differences between inputs are amplified, whereas common signals are rejected in a process called common mode rejection (CMR). In order for CMR to be most effective in reducing noise, the electrode impedances should be balanced. Filters are part of the differential amplifier as they remove unwanted noise of a certain frequency. The frequency bandwidth, or passband, is the range of frequencies between low- and high-frequency filter settings that are not filtered out. Major sources of noise that cause interpretation artifacts are power lines, amplifier noise, physiologic activity, electrochemical electrode noise and circular grounds. Noise reduction is achieved with high amplifier input impedance, balanced electrode impedances, CMR, filters and signal averaging. Maintaining electrodes in good condition, achieving proper contact between electrode and animal, and keeping electrode leads short aid in achieving noise reduction.

Key Words: common mode rejection, differential amplifier, electrode, filter, impedance, passband

INTRODUCTION

Electrodiagnostic techniques are valuable tools in clinical and investigative veterinary ophthalmology used to assess the functional integrity of the retina, the optic nerve and the central visual pathways by recording and displaying cell membrane potential changes. The most commonly used electrodiagnostic methods in clinical veterinary ophthalmology are the flash electroretinogram (ERG) and flash visual-evoked potentials (VEPs).1 Other methods that have been reported by veterinary ophthalmologists include pattern2 and multifocal ERG3 as well as the electrooculogram (EOG).4 These methods differ mainly in the stimulus used to elicit a response and the analysis of the recorded data. This article reviews some of the basic principles of recording that are shared by all of these techniques. The recording of signals without artifacts can be challenging. This article should help the veterinary ophthalmologist to improve his or her recording techniques by finding and eliminating the sources of recording artifacts.

Electrodiagnostic techniques record the sum of cell membrane potential changes over time in a large number of excitable cells in the vicinity of the electrodes in response to visual stimulation. The magnitude and polarity of the
electric current is defined as charge moved per unit time. If

disease-related electrophysiologic changes (Fig. 1). can result in recordings from normal eyes that are similar to
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neural visual system can be disturbed by disease or injury leading
to altered recorded electrophysiologic potentials. Interpretation
of these electric changes can be of important diagnostic and prognostic value, although it is important to be aware of
technical factors and the limitations of the techniques which
can result in recordings from normal eyes that are similar to
disease-related electrophysiologic changes (Fig. 1).

DIRECT AND ALTERNATING CURRENTS

Electric current is defined as charge moved per unit time. If
current flows in only one direction, whether steadily or in

recorded potential may be influenced by equipment-related factors (distance and relative position of the electrode from
the site of membrane potential changes, conductor character-
istics of the electrodes, filter and amplifier settings, and stimulus-related parameters such as brightness) and animal-
related factors (presence of disease, level of dark-adaptation,

1 Hz = 1 s
frequencies, or numbers of cycles per second, in Hertz (Hz,
1 Hz = 1 s⁻¹).

Table 1 Important physical relationships

<table>
<thead>
<tr>
<th>Expression</th>
<th>Formula</th>
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<tbody>
<tr>
<td>Ohm's law for direct current (DC): current</td>
<td>$\text{current} = \frac{\text{voltage}}{\text{resistance}}$</td>
</tr>
<tr>
<td>Ohm's law for alternating current (AC): current</td>
<td>$\text{current} = \frac{\text{voltage}}{\text{impedance}}$</td>
</tr>
<tr>
<td>Gain of an amplifier: gain</td>
<td>$\text{gain} = \frac{\text{voltage after amplification}}{\text{voltage before amplification}}$</td>
</tr>
<tr>
<td>Common mode rejection ratio (CMRR) of an instrumentation amplifier: CMRR (in decibels, dB)</td>
<td>$\text{CMRR} = 20 \times \log \left( \frac{\text{gain of biological signal}}{\text{gain of 60 Hz noise}} \right)$</td>
</tr>
<tr>
<td>Voltage at the amplifier input ($V_A$) as a function of original voltage ($V_k$), amplifier input impedance ($Z_{in}$), and electrode impedance ($Z_e$, replaced by $Z_{G1}$, $Z_{G2}$, or $Z_{GND}$ from Fig. 2):</td>
<td>$V_A = V_k \times \left( \frac{Z_{in}}{Z_{in} + Z_e} \right)$</td>
</tr>
</tbody>
</table>

Units: current in A, voltage in V, resistance and impedance in Ω.
INSTRUMENTATION

Most modern clinical electrodiagnostic equipment is user-friendly, with only a few settings that need to be adjusted. Nevertheless, it is important to keep certain basic principles in mind for recording and interpreting bioelectric signals correctly.

An active (positive), a reference (negative) and a ground electrode are used on the animal to record bioelectric signals (Fig. 2). Active electrodes to record retinal potentials on the cornea include mono- and bipolar contact lens electrodes, fibers and gold plates (Fig. 3). Monopolar contact lens electrodes only have one lead touching the cornea, whereas bipolar contact lens electrodes contain a second reference lead touching the eyelids. The advantage of a fiber electrode is that it will float in the tear film, will not change the optical properties of the cornea, and, in general, does not require any topical anesthetic or ionic conductive solutions. However, in the anesthetized animal the cornea is not protected and needs to be covered by a contact lens or by frequent applications of an artificial tear solution. Contact lens electrodes, in contrast, do protect the corneal surface. These electrodes require the application of topical anesthetic (if no general anesthesia is used) and a ionic conductive solution for proper contact with the cornea. Unless custom-built for a particular animal, the contact lens curvature differs from the corneal curvature and it will therefore change the optics of the eye. Consideration of the resulting refractive error is important for the recording of pattern and multifocal ERGs, where the stimulus needs to be in focus on the retina. The gold rim that is found on many commercial contact lens electrodes can act as an artificial pupil and affect the amount of light that enters the eye. The property of any ionic conductive solution is very important. Solutions of too high viscosity (i.e. more viscous than 0.5% methyl cellulose) can attenuate signal amplitudes.

Two basic types of skin electrodes are used as reference or ground electrodes to record ERGs and VEPs, and also as active electrodes to record VEPs: surface and needle electrodes (Fig. 4). Electrodes are made of metals such as stainless steel, platinum, silver-silver chloride, nickel chromium, and silver and gold alloys and plating.

The recorded potential represents the difference of voltage between the active and reference electrode. The ground electrode serves as zero, which means that the polarity of the recorded voltage is positive or negative relative to ground (Fig. 2).
The following placement of the electrodes is commonly used and recommended for the recording of ERGs in animals:1,10–12 active electrode on the cornea, reference electrode between lateral canthus and ear (at least 2 cm from the active electrode), and the ground electrode on the pinna of the ear or over the external occipital protuberance. The following electrode placement seems to be most appropriate for the recording of VEPs: active electrode 1–3 cm rostral to the bregma and the negative electrode under the chin.1 The location of the ground electrode is less critical and can be in the interscapular area.

An amplifier used to record biological signals enhances the signal of interest, depending on the original amplitude, from 10 to several 100 000 times, while rejecting unwanted signals or noise. The amplified signal should be in the order of 1–10 V so that it can be detected by the electronic circuit that is responsible for displaying the signal. The gain of an amplifier is defined in Table 1.

An amplifier that selectively amplifies signals of interest while rejecting noise is called a differential or instrumentation amplifier. Noise will appear similarly at both the active and reference electrode if the two are placed reasonably close together on the animal. Signals that are common to both inputs will not be amplified but eliminated (Fig. 2). The active electrode is placed closer to the signal source of interest, whereas the reference electrode is placed a little bit farther away where it is still exposed to background noise but not to the targeted tissue signal. The signal of interest represents the difference between the active and reference inputs and is amplified (Fig. 2). The process of amplifying the difference between inputs while eliminating the common signal is called common mode rejection (CMR). Any signal in proximity to the reference electrode (e.g. muscle activity) will also be amplified if it does not occur at the active electrode and will mask the signal of interest. Common mode rejection is quantified in technical specifications of amplifiers as CMR ratio (Table 1).2

To measure potential changes, current must flow through the electrode into the amplifier (input) and return to the animal through the ground lead. Because of the electrode impedance, a voltage drop occurs. The voltage seen by the amplifier is dependent on the voltage before the electrode, the electrode impedance, and the amplifier input impedance (Fig. 2, Table 1). The amplifier impedance has to be much greater than the electrode impedance (at least 100 times greater) to minimize a voltage drop.13

In order for CMR to be able to eliminate background noise, the active and reference electrode impedances (voltage drops) should be similar. If these impedances differ, noise will be attenuated differently by the electrodes. Since the difference between the two inputs is amplified, there will thus be more noise in the recorded signal wavelength.

In general, a recorded biological waveform consists of high- and low-frequency signal elements. A Fourier analysis may be used to describe the frequency components in a recorded signal. However, it is rarely utilized in clinical applications. The electrophysiologist’s goal is to record all frequencies that are part of the biological potential changes and suppress any unrelated frequencies or noise because they distort the waveform and potentially lead to false interpretation. Filters are part of a differential amplifier as they remove unwanted noise of certain frequency. Low-frequency, or high-pass, filters remove low-frequency signals or noise. High-frequency, or low-pass, filters remove rapidly changing, or high-frequency, components. The frequency bandwidth, or passband, is the range of frequencies between the low- and high-frequency signal elements. A Fourier analysis thus be more noise in the recorded signal wavelength.

The frequency at which half of the signal is removed is the cut-off frequency. The roll-off of a filter reflects how rapidly the amplitude attenuates as the frequency changes beyond the cut-off value. Filters allow visualization of small signals that are usually not clearly visible in the displayed waveform (Fig. 6). Noise can be easily removed if its frequency is sufficiently different from frequency components of the bioelectric signal of interest. If noise and signal consist of similar frequencies, filtering of noise will also filter parts of the signal and distort the resulting waveform. This is often the case for 60 or 50 Hz noise from power lines because many biological signals contain components of these frequencies.

The recorded waveform is either displayed analogically by the cathode ray tube of an oscilloscope, or it is digitized by an analog-to-digital converter and displayed digitally. The horizontal setting of the waveform display is referred to as sweep and the vertical setting as sensitivity.

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SOURCES OF NOISE

The major sources of noise are power lines, amplifier noise, physiologic activity that is not relevant, electrochemical electrode noise and circular grounds. Depending on the geographic location, power lines carry sine wave currents with either 110 V/60 Hz (e.g. USA) or 220 V/50 Hz (e.g. Europe). Small power line interference noise flows into the animal, the amplifier circuits, and/or the recording leads through capacitative and inductive coupling.14

Internal, thermal amplifier noise is usually spread over a wide frequency range. It often appears as fluctuations, or fuzziness, in the baseline and is described in the technical specifications of a particular amplifier.

Background electrophysiologic activity (e.g. skeletal or heart muscle activity) can disturb the waveform being studied, especially if it occurs in the proximity of only one electrode such that it will not be eliminated by CMR. Because the active electrode is placed close to the location of interest, it is usually the reference electrode that is closer to the noise source. If the animal is not sufficiently relaxed, excessive muscle activity will show up as noise on the display (Fig. 7).

Electrode potentials develop at the surface of an electrode where it is in contact with electrolytes from electrode paste, sweat or extracellular fluid. Changes in these electrode potentials are a source of noise (electrochemical electrode noise). Such changes occur when electrodes move or when electrodes are dirty, corroded (e.g. poor quality electrodes) or the coating is thinned.

Multiple grounding of the electrodiagnostic equipment creates ground loops in which noise currents can circulate (circular grounds).

NOISE REDUCTION

The goal of electrodiagnostic recording is to maximize the signal of interest and reduce background noise. Noise reduction improves the quality of the waveform and makes clinical interpretation easier and more accurate. Some of this noise is removed by CMR of the amplifier, but if a current flows between the active and reference electrode the noise will not appear the same at the amplifier inputs and will be amplified. High amplifier input impedance (relative to lower electrode impedance), balanced electrode impedances, CMR and filters eliminate most of the noise.

In most cases of excessive noise in the recorded waveform, the impedances of the active and reference electrodes are not matched. Signal amplitude is enhanced and noise is lessened by reducing and matching the electrode impedances. Maintaining the electrodes in good condition (clean and free of corrosion), achieving proper contact between...
electrode and animal (e.g. sufficient amount of ionic conductive solution without air bubbles between cornea and contact lens, proper skin preparation), and keeping electrode leads short to reduce inductive coupled interference aid in achieving noise reduction. To achieve the latter, the amplifier should be in close proximity to the animal. Unmatched electrode impedances can occur due to breaks in the electrode lead.

Electrochemical electrode noise is kept low by keeping the electrodes in good condition and by using high-quality electrodes such as silver–silver chloride electrodes that have stable electrode potentials and are therefore relatively noise free.

Unnecessary electric equipment in the examination room should be turned off because it is a potential source of power line interference. Recordings should be performed with the animal in different locations in the room to identify sources of noise and to find the quietest area. Power line interference is highest near electric equipment and power lines in the wall. Changing the orientation of the amplifier and electrode leads can help minimize capacitive and inductive coupling. If the noise persists, recordings should be done in a different room. The alternative consists of shielding the examination area from sources of power line interference with surrounding, grounded metal plates or wire meshworks. No equipment run on AC power should be used within such a shielded area. Because most signals of clinical interest contain components near 60 Hz frequency, a notch filter to remove 60 Hz noise originating from AC power lines should be used sparingly, as distortion of the waveform may occur. Circular grounds are avoided by grounding all the electrodiagnostic equipment to one point.

If relocation of the reference electrode does not eliminate background electrophysiologic noise, other measures have to be considered, such as deeper sedation or anesthesia of the animal in case of excessive skeletal muscle activity. Most animals need to be under general anesthesia for proper recording of ERGs. One exception might be the rapid evaluation of retinal function in dogs under sedation prior to cataract surgery. Early signs of retinal disease can easily be missed using this latter method. Although deep sedation or general anesthesia is recommended for proper recording, one should be aware of the effects that various anesthetics have on the function of the retina and the central visual pathway as shown by numerous studies. As an example, Acland et al. showed a significant effect of halothane on the amplitude, timing and waveform of the poodle electroretinogram. Lalonde et al. recently showed in pigs that isoflurane mimics the effect of optic nerve axotomy in the flash, pattern and multifocal ERG.

Background noise is reduced by the low- and high-pass filters. The filter setting should minimize the noise without distorting the signal. In addition, signal averaging can be used to enhance the signal of interest while minimizing nonsynchronous noise. Whenever a stimulus occurs (e.g. light flash or pattern reversal) a trigger is sent to the recording system to register the response of the animal. The trigger must be at a fixed time relative to the stimulus pulse so that a particular component of the waveform occurs at exactly the same time in each sweep of the series. Because the noise occurs randomly in relation to the trigger, it will be reduced, whereas the signal will be maintained because of its set time relationship with the trigger. The use of signal averaging should not replace careful control of stimulating and recording conditions. If averaging is used inappropriately it can interfere with the adaptation state of the retina.

ANIMAL SAFETY

Electric equipment, including electrodiagnostic and anesthetic instruments, is powered by either 110 V/60 Hz or 220 V/50 Hz AC. Within the instrument the AC power is transformed in low-voltage DC current that supplies the amplifiers and other parts of the equipment. The wall outlets that are used to power electric equipment consist of three wires – one active, one neutral connected to earth ground, and a third safety ground also connected to earth ground. The high-voltage parts of the instrument are isolated from the animal and the user to prevent physical contact.

In older instruments, despite the isolators mentioned above, leakage currents may still flow because of capacitive coupling to other parts of the instrument such as the chassis. Defective isolators also allowed the flow of leakage currents. There was a potential risk that an animal or equipment user could be shocked by these leakage currents. If the grounded animal came into contact with the chassis of the instrument, the leakage current would flow through the animal because this represented the only pathway to ground. The leakage current could induce ventricular fibrillation. Leakage current could originate from any electrical equipment (e.g. heating blanket) with a missing or broken safety ground that was touching the grounded animal.

The risk for the patient of being exposed to leakage currents is reduced by connecting a low-resistance ground wire to the equipment chassis so that leakage current will not flow through the animal. Standards have been established for the maximum allowable leakage currents. Based on these standards, the animal is electrically isolated from power lines and earth ground by optical isolation or capacitive or inductive coupling in newer amplifiers. In optical isolation the electrophysiologic signal is converted into an optical signal that is transmitted across the electric barrier and then reconverted into an electric signal. Even if the animal is in direct contact with a high-voltage power line, it is unlikely that current could flow through the organism to ground if the equipment is optically isolated.

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