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Electroretinogram as a diagnostic tool in veterinary practice

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Abstract
The cranial cruciate ligament or anterior cruciate ligament is the ligament that usually tears in dogs and more frequently seen in overweight, neutered, middle-aged dogs. There are two ligaments within the knee joint that form a cross or x-shape, thus the name cruciate ligaments. The ligaments do not have a good blood supply and no mechanism for repairing themselves. For diagnosis of cranial cruciate ligament tears or ruptures, acute lameness with the characteristic "toe-touching" gait or as a chronic lameness may be an important sign but for confirmatory diagnosis Cranial Drawer Test is mostly done with radiographic examination. There are a number of non-surgical and surgical treatment options are available.

Keywords: Cruciate, ligament, gait, rupture

1. Introduction
Vision, the capability we have of seeing something, is not as simple as you might expect. In fact, it is a result of the complex system of the eye, involving many processes. The main principle behind the functioning of the eye is that of refraction, which occurs four times between the instant a light ray reaches your eye and the moment an image is registered on the retina.

In dogs, as in many other animal species, subjective assessment of retinal function is not readily available. Various clinical and experimental studies have proven that the ERG is an effective and objective method of assessing retinal function [1-5]. Electroretinography measures the electrical responses of various cell types in the retina, including the photoreceptors (rods and cones), inner retinal cells (bipolar and amacrine cells), and the ganglion cells. Electrodes are usually placed on the cornea and the skin near the eye, although it is possible to record the ERG from skin electrodes. During a recording, the patient's eyes are exposed to standardized stimuli and the resulting signal is displayed showing the time course of the signal's amplitude (voltage). Signals are very small, and typically are measured in micro volts or nano volts. The ERG is composed of electrical potentials contributed by different cell types within the retina, and the stimulus conditions (flash or pattern stimulus, whether a background light is present, and the colors of the stimulus and background) can elicit stronger response from certain components.

If a flash ERG is performed on a dark-adapted eye, the response is primarily from the rod system. Flash ERGs performed on a light adapted eye will reflect the activity of the cone system. Sufficiently bright flashes will elicit ERGs containing an a-wave (initial negative deflection) followed by a b-wave (positive deflection). The leading edge of the a-wave is produced by the photoreceptors, while the remainder of the wave is produced by a mixture of cells including photoreceptors, bipolar, amacrine, and Muller cells or Muller glia. [1] The pattern ERG, evoked by an alternating checkerboard stimulus, primarily ref;ects the activity of retinal ganglion cells. Inherited retinal degenerations in which the ERG can be useful include Retinitis pigmentosa and related hereditary degenerations, Retinitis punctata albescens, Leber's congenital amaurosis, Choroideremia, Gyrate atrophy of the retina and choroid, Goldman-Favre syndrome, Congenital stationary night blindness - normal a-wave indicates normal photoreceptors; absent b-wave indicates abnormality in the bipolar cell region, X-linked juvenile retinoschisis, Achromatopsia, Cone dystrophy, Disorders mimicking retinitis pigmentosa and Usher Syndrome. Other ocular disorders in which the standard ERG provides useful information are Diabetic retinopathy, some other ischemic retinopathies including central retinal vein occlusion (CRVO), branch vein occlusion (BVO), and sickle cell

retinopathy. Toxic retinopathies, including those caused by Plaquenil and Vigabatrin. The ERG is also used to monitor retinal toxicity in many drug trials. The Retinal detachment and assessment of retinal function after trauma, especially in vitreous hemorrhage and other conditions where the fundus cannot be visualized it may be a useful diagnostic aid.

The ERG is also used extensively in eye research, as it provides information about the function of the retina that is not otherwise available. Other ERG tests, such as the Photopic Negative Response (PhNR) and pattern ERG (PERG) may be useful in assessing retinal ganglion cell function in diseases like glaucoma. The multifocal ERG is used to record separate responses for different retinal locations.

Electroretinography (ERG) has proven to be an objective and a useful tool to assess retinal function in animals. The technique is most commonly used for a pre-operative evaluation of retinal function in association with cataract surgery. It is also used for diagnostics in association with various forms of retinal degenerations such as progressive retinal atrophies (PRA), sudden acquired retinal degenerations (SARD) and toxic retinal degenerations. In recent years ERGs have been invaluable in assessing the effect of treatment trials for retinal degenerative disease, such as gene therapy, stem cell therapy and sub retinal implants [1, 2]. It is important to keep in mind the possibility of recording a perfectly normal ERG from a completely blind patient and that the only information obtained by the ERG is whether the retina is functional or not.

In order to investigate the visual pathway and/or cortical function other diagnostic tests such as recording of visual evoked potentials (VEP) are needed. The flash ERG and flash visual evoked potentials (VEPs) are the most commonly used electrophysiological techniques in veterinary ophthalmology.

In 1989 a basic protocol was standardized so that ERGs could be recorded comparably throughout the world [1]. This standard was updated most recently in 1999 [2]. Standards for five commonly obtained ERGs were presented:

- ERG to a weak flash (arising from the rods) in the dark-adapted eye
- ERG to a strong flash in the dark-adapted eye
- Oscillatory potentials
- ERG to a strong flash (arising from the cones) in the light-adapted eye
- ERGs to a rapidly repeated stimulus (flicker)

Other electrophysiological methods such as the electro-oculogram (EOG), pattern and multifocal ERG are important but less frequently used diagnostic tools.

2. The Flash Electroretinogram

The full field ERG is a summation of electrical impulses obtained after stimulation of the entire retina with light [3]. By altering the conditions under which the ERG is recorded as to the intensity, frequency, wavelength and duration of the light stimulus, in addition to the state of light adaptation of the patient, the function of the different retinal cells can be evaluated individually. A stimulation system with a blue-white xenon flash of light has often been used to stimulate the retina, but newer recording equipments with Light Emitting Diodes (LED) is becoming more common [4]. Visual stimulation of the retina with light induces membrane potential changes over time in a large number of excitable cells [5]. Thus, electrical currents are produced and recorded by means of electrodes.

The electrical activity picked up by the electrodes is processed by a computer and Electroretinograms are obtained. Several technical and biological factors may influence the outcome of these electrophysiological recordings.

3. Biological Factors

Biological aspects which may influence the results of the ERG recordings are: age of the patient, breed, time of the day, prior exposure to bright light, pupil dilation, position of the eye. The position of the patient, distance between patient and stimulator, choice and depth of sedation/anaesthesia, drugs, body temperature and oxygenation [6-11]. It is important to use standardized conditions when performing ERGs in order to be able to evaluate the recordings in a correct manner. ERGs should preferably be performed at the same time of day due to diurnal variations in the "shedding" of the photoreceptors [9]. Pupil dilation, position and exposure of the eye should be assessed by means of dim red light prior to and during the ERG-procedure. In order not to light adapt the rod photoreceptors' exposure to bright light, such as when performing fundus photography and indirect ophthalmoscopy, should be avoided at least one hour prior to ERG examination [12]. Downward rotation of the eye can be avoided by the use of conjunctival stay sutures, muscle relaxant drugs or retro bulbar saline injections. Further, eyelid speculae ensure good separation of the eyelids.

4. Electrodes

In order to record an ERG an active (positive) corneal electrode is used, a reference electrode (negative), positioned 2-5 cm temporal to the lateral canthus of the eye, and a ground electrode, usually placed at the occipital process [13, 14]. A variety of corneal electrodes are available, including mono- and bipolar contact lens electrodes, fibers and gold plates. The monopolar contact lens, such as the Jet lens, is the most commonly used in canine ERGs. An ionic conductive solution, such as methylcellulose or a carbomer gel, is used for proper contact with the cornea. It is important to avoid air bubbles between the contact lens and the cornea in order to obtain reliable results. Fibre electrodes may also be used, such as the Dawson, Trick, and Litzkow (DTL) electrode. Reference and ground electrodes can be epidermal (surface electrodes) or intra dermal (needle electrodes) made of metal, such as platinum, silver-silver chloride, nickel chromium, stainless steel, silver and gold alloys and plating. To allow comparison between ERG recordings in and between patients, the electrodes and their positions have to be standardized as variations in these parameters significantly influence the results obtained [14].

5. ERG Protocol

The choice of ERG protocol is dependent upon the objective for the examination. If the main goal is to evaluate whether a patient is suitable for cataract surgery or not, or if it is suffering from SARD, a short ERG protocol can be sufficient, while if the aim of the examination is to characterize or differentiate a generalized retinal degeneration, a more meticulous procedure should be considered. Detailed information on recommended protocols for the dog is given in "Guidelines for clinical electroretinography in the dog" by Narfström *et al.* [13]. Based on these protocols it is possible to add additional tests if needed, although care must be taken, especially under scotopic conditions, not to light adapt the retina. Rod function is investigated following dark adaptation

using low intensity stimuli. The mixed rod-cone function is also investigated under scotopic conditions using higher stimuli. Pure cone derived recordings can be achieved by light adapting the retina for ten minutes to bright light (30-40 cd/m2), due to desensitization of the rod system. Cone function is thereafter studied using multiple fl ashes of bright light. The critical flicker fusion frequency (CFFF) is the frequency of light stimulation using a specific intensity of light where the eye can no longer discern flickering light as single fl ashes, but perceives a steady light [15]. The CFFF varies between species and with background light intensity. High light intensity flicker stimulation can be used to separate cones from rods since the CFFF is much lower for rods (10-20 Hz) than for cones (60-90 Hz) for the canine specifically [16].

The "standard" full field ERG using bright light in scotopic conditions is composed of a leading negative a-wave followed by a positive b-wave. A positive c-wave can be observed under very stable recording conditions when longer duration fl ashes are used (>300 ms) [17]. The a-wave originates mainly from the photoreceptors, the b-wave from the ON-bipolar cells and the c-wave from the retinal pigment epithelium (RPE). In addition, several other wavelets such as the i-wave and d-wave can be observed under certain conditions. Amplitudes, implicit times, wave forms and whether specific wavelets are present or not in the recording, vary between species [18, 19].

Oscillary potentials (OPs) can be seen on the rising phase of the scotopic and photopic b-wave under certain circumstances and are considered to mainly represent amacrine cell contributions. Rod- and cone photoreceptors contribute to the ERG to varying degrees, depending on light adaptational status of the retina and the intensity of the stimulus used. In general, rod photoreceptors dominate in the dark adapted retina (scotopic conditions) using low intensity stimuli, while the cone photoreceptors contribute significantly in the light adapted retina (photopic conditions) using stimuli of higher intensity. Mixed rod-cone activity can be investigated using higher intensity stimulus under scotopic conditions. The ERG recordings obtained under photopic conditions using high intensity flickering light is mainly cone photoreceptor mediated, although other retinal cells, such as bipolar cells, contribute to a varying degree depending on the frequency and duration of the light flashes [5].

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