The ERG of the Beagle dog: evidence associating a post b-wave negativity with the Tapetum Lucidum

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Abstract

As previously reported in the literature, the electroretinogram (ERG) of the Beagle dog includes a large post b-wave negativity, the origin of which is not yet established. In the course of our investigations on the electroretinogram in dogs, we examined two Beagle dogs (2 years apart) who had one eye devoid of a Tapetum Lucidum (TL). Photopic (cone-mediated) and scotopic (rod-mediated) ERGs were obtained according to the guidelines for clinical electroretinography in dog. In both dogs the short-latency ERG components (i.e. a- and b-waves) were found to be within the normal range in amplitude, peak time and morphology O.U. However, the large negative component that, in Beagle dogs, normally follow the b-wave was absent from the photopic as well as the scotopic signals obtained from the TL-free eye. Our results thus suggest a possible contribution of the TL to the ERG of Beagle dogs.

Introduction

It has always been our experience that the ERG of the Beagle dogs included a strong negative component that followed the b-wave, irrespective of the state of retinal adaptation [1, 2]. Given that the analysis of the ERG is usually limited to the early components (a-wave, b-wave and oscillatory potentials), we had never really paid attention to this negative wave. Similarly, although a negative post b-wave component could also be seen in recordings illustrated in other publications reporting on the ERG of Beagles [3 (figure 6), 4 (figures 2 and 5)], poodles [5 (figure 2)] and Briards [6 (figure 3A, photopic single sweep), 7 (figure 3, control photopic response)] for example, only Schaeppi and Liverani [4] commented on it. They showed that this negative component was most prominent in light adapted signals. However, it does not appear to be an universal feature of the retinal signal since there are also published materials, reporting on the electroretinogram of dogs, that do not include this negative component [8]. It could be that the stimulus parameters that are optimal for this ERG component are not yet known.

In the course of our investigations on the electroretinogram in Beagle dogs, we examined two different dogs (at two years interval) with one eye devoid of a Tapetum Lucidum (TL), the structure which is responsible for the “eyeshine” seen at night or under reduced illumination such as during a fundus examination. Of interest, the tapetum-free eyes did not generate the above-mentioned post b-wave component, suggesting the participation of the TL to its genesis.
Material and methods

Subjects

As part of our ongoing research projects on the ERG of the Beagle dogs, we identified (at 2 years apart) two male Beagle dogs, aged 2 and 2.5 years old, that presented with a unilateral absence of the Tapetum Lucidum. Following a complete physical examination, the dogs were found to be otherwise healthy. A complete ophthalmological assessment, including fundus photography (Kowa RC-2, Luneau, France) and a behavioral evaluation of the visual function (such as menace and “paper ball” reflexes) were conducted 72 h prior to the ERG recordings. For the ERG recordings, the pupils were maximally dilated with drops of tropicamide (Mydriaticum, MSD, Paris, France) in each eye to obtain a stable mydriasis. The dogs were then anesthetized with a single intramuscular injection of a mixture of ketamine (5 mg/kg, Imalgene, Mériel, Lyon, France) and medetomidine (0.04 mg/kg, Domitor, Pfizer, Orsay, France). Rectal temperature, blood pressure and heart rate remained within the normal physiological limits throughout the experiment as measured with the Finapres 2300 system (Ohmeda SA, Trappes, France). Measurements of the intraocular pressure (Tonopen, Luneau, Paris, France) were also obtained at the beginning and the end of the recording sessions.

Material

A Visiosystem (Siem Bio-Médicale, Nîmes, France) was used to generate the flash stimuli as well as perform the recordings and analysis the ERG responses. Binocular ERGs were evoked with the use of two bright flashes of light (Xenon capacitive discharge adjustable photosimulators; maximum intensity: 6.5 cd s/m²; duration: 20 μs) of 4 cm in diameter, each covered with a frosted glass diffuser and positioned at 1.5 cm from each eye in order to achieve a full field condition [1, 9]. As previously reported [10], immobilization of the head was obtained with the use of a specific contention device and the eyes were maintained opened and the third eyelid prolapsed, with the use of sterile sclero-conjunctival copper clips attached to the conjunctiva of the superior part of the globe. These clips also served as the active ERG electrodes. Reference and ground electrodes were inserted subcutaneously at the level of the outer canthus and neck respectively (sterile acupuncture needles of 0.2 mm in diameter manufactured by Asiamed, Paris, France). Corneal hydration was maintained throughout the entire procedure with the use of a viscoelastic gel (Ocrygel®, Laboratoire TVM, Limoges, France). The ERG responses were amplified 10,000 times within a 0.1–300 Hz recording bandwidth (6 db per octave; 50 Hz notch filter) and recorded over a 250 ms period.

Procedure

According to the guidelines for clinical electroretinography in dog [2], the retinal signals were obtained in photopic and scotopic conditions. The rod desensitising background was obtained with the use of three daylight ceiling fluorescent lights which provided a photopic environment of 30 cd/m² measured at the level of the dogs’ eyes. Following a preadaptation period of 3 h to this photopic environment (done on awake animals in order to acclimatize them to the laboratory surroundings), the dogs were prepared as described above. Then, an average of 5 white bright flashes (intensity: 2.5 cd s/m²), was delivered at 1.3 Hz. The ERG recorded was representative of the photopic (cone-dominated) responses. The rod desensitizing background lights were then turned off and after a 30 minute-adaptation to dark, an average of 3 flashes of dim blue light [Kodak Wratten filter 98 (440 nm); 0.025 cd s/m²] was delivered at 0.1 Hz; the ERG recorded was representative of the scotopic (rod-dominated) response. Then, a single bright flash (white light; intensity: 2.5 cd s/m²) was delivered in the dark and the ERG thus obtained was taken as representative of the mixed rod-cone response. The entire procedure lasted 30 min approximately. For one dog this procedure was repeated on three different occasions, spaced by a one month interval in order to assess the reproducibility of our observation. Necropsy was performed in one dog. The eyes were enucleated and the retinas were prepared for histology. Retinal sections were stained with hematoxilin–eosin.