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A new DTL-electrode holder for recording of electroretinograms in animals

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ABSTRACT

Purpose: Contact lens electrodes (CLEs) are frequently used to register electroretinograms (ERGs) in small animals such as mice or rats. CLEs are expensive to buy or difficult to be produced individually. In addition, CLE's have been noticed to elicit inconstant results and they carry potential to injure the cornea. Therefore, a new electrode holder was constructed based on the clinically used DTL-electrode and compared to CLEs.
Material and methods: ERGs were recorded with both electrode types in nine healthy Brown-Norway rats under scotopic conditions. For low intensity responses a Naka–Rushton function was fitted and the parameters V_{max}, k and n were analyzed. The a-wave, b-wave and oscillatory potentials were analyzed for brighter flash intensities (1–60 scot cd s/m^2). Repeatability was assessed for both electrode types in consecutive measurements.
Results: The new electrode holder was faster in setting up than the CLE and showed lower standard deviations. No corneal alterations were observed. Slightly higher amplitudes were recorded in most of the measurements with the new electrode holder (except amplitudes induced by 60 cd s/m^2). A Bland–Altman test showed good agreement between the DTL holder and the CLE (mean difference 35.2 μV (Holder-CLE)). Pearson's correlation coefficient for test–retest-reliability was $r = 0.783$.
Conclusions: The DTL holder was superior in handling and caused far less corneal problems than the CLE and produced comparable or better electrophysiological results. The minimal production costs and the possibility of adapting the DTL holder to bigger eyes, such as for dogs or rabbits, offers with broader application prospects.

1. Introduction

Assessment of retinal function is essential in a variety of clinical and research settings (DiLoreto et al., 1995; Messias et al., 2010). Electroretinography (ERG) is without doubt currently the leading technique to objectively describe retinal function. It has been used successfully for decades (Kahn and Löwenstein, 1924; Karpe, 1945) since it is noninvasive, well tolerable, and can be easily repeated in longitudinal studies (Sauve et al., 2006; Rosolen et al., 2008). Sophisticated design of stimulation and recording parameters and detailed interpretation of signals can allow assessment of global retinal function and even of single cell layers (Dalke et al., 2004; Messias et al., 2008b). Methodology for animal examinations is fairly well established (Cone and Platt, 1964; Peachey and Ball, 2003; Pinilla et al., 2004), although not uniformly applied. ERG electrodes used to register signals from the anterior segment of the eye (mostly the cornea) differ considerably (Bui et al., 1998; Mentzer et al., 2005; Yamashita et al., 2009). For small animals such as rats and mice ERG electrodes are frequently developed individually by the laboratories (Rosner et al., 1993; Sagdullaev et al., 2004). Very often contact lens electrodes (CLEs) are used which have shown to evoke good electrophysiological responses (Bayer et al., 1999).

However, in our experience CLEs possess several potential shortcomings. First, CLEs are often either expensive to acquire or they have to be constructed and manufactured by the laboratories themselves. Manufacturing requires sophisticated machinery in order to achieve a good enough surface to smoothly contact the cornea and also to contain the electrode material. Inert electrode material such as gold or platinum is expensive. A second disadvantage of CLEs is that they possess an inherent potential to irritate the cornea and cause erosions, even when sophisticated manufacturing techniques such as computer-driven lathing ensure smooth surfaces. Erosions can lead to acute and transient or even permanent corneal haze. Haze then alters light transmission and makes ERG signals unpredictable. Certainly, corneal injury can partly be avoided by careful electrode handling. But in order to make ERG recording more practicable and also more widely available attaching and re-adjusting electrodes should be designed as easy and safe

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as possible, especially since this manoeuvre often takes place in the dark. In our personal observation, even though computer aided design and lathing is used to ensure very smooth surfaces, various amounts of corneal injury and haze are seen in up to 10% of rats. A third potential disadvantage is that polymethyl methacrylate (PMMA), which is probably most often used for CLEs, has a tendency to become matt after frequent use and especially after sterilization with alcohol containing substances. This results in a frosted appearance that can alter light transmission just as corneal haze. But even clear and transparent CLEs alter transmission of light and can in the worst case be a barrier for certain wavelengths used for specific testing.

Although we overall have had good experiences and registered good and stable results with our CLEs (Thaler et al., 2008), these potential disadvantages prompted us to investigate new possibilities for ERG recording electrodes that could improve results and protect the animals' corneas. One obvious possibility was an electrode holder which offers the possibility to measure ERG in animals with the DTL-electrode (Dawson–Trick–Litzkow electrode (Dawson et al., 1979)) commonly used in clinical settings in humans. Therefore, we designed a new DTL-electrode holder and tested ERG recordings against our standard CLEs. In addition, handling parameters of the two electrodes were compared.

2. Methods

2.1. Animals

Nine healthy Brown Norway rats (Charles River Laboratories GmbH, Sulzfeld, Germany) with a mean weight of 178.79 g ± 8.48 g (mean ± standard deviation (SD)) at baseline were included. The experimental procedure was performed following the recommendations of the ARVO statement for the use of animals in ophthalmic and vision research. The rats were housed under standard laboratory conditions with light-dark cycles of 12 h/12 h.

2.2. Electroretinography

The ERG was measured after a period of 12 h dark adaptation. Rats were anesthetized with a combination of ketamine 100 mg/kg and xylazine 5 mg/kg (WDT eG, Garbsen, Germany) injected intraperitoneally. To dilate the pupils 1 drop of tropicamide (Mydriatikum® 0.5%) was applied 10 min before the ERG measurement started. One drop of Methocel® eye-gel was applied to improve the contact between electrode and cornea before the CLE was placed in one and the DTL-electrode holder in the other eye as active electrodes. Two subcutaneous needle-electrodes (Ambu® Neuroline Twisted Pair Subdermal, Bad Nauheim, Germany) one inserted between the eyes and the other in the tail served as reference and ground electrodes, respectively. Electrodes were attached such that an acceptable impedance level of <10 kΩ at 25 Hz (manufacturer's recommendation) was ensured before and during the measurement.

The ERG protocol consisted of 16 steps with raising intensities from 0.000003 to 60 scot cd s/m^2 which were produced by a mixed light (white 6500 K) with a Ganzfeld stimulator (ColorDome®, Diagnosys LLC, Cambridge, GB). The duration of flashes was fixed at 4 ms. All flashes were delivered without background illumination and constant inter-stimulus-intervals of 1 s for dim flashes and up to 45 s for bright flashes were used to continuously ensure stable dark adapted conditions. Band-pass filtering was applied from 0.3 to 300 Hz using the machine's built-in software algorithm. Averages ranged from 20 trials for dim flashes to 2 trials for bright flashes.

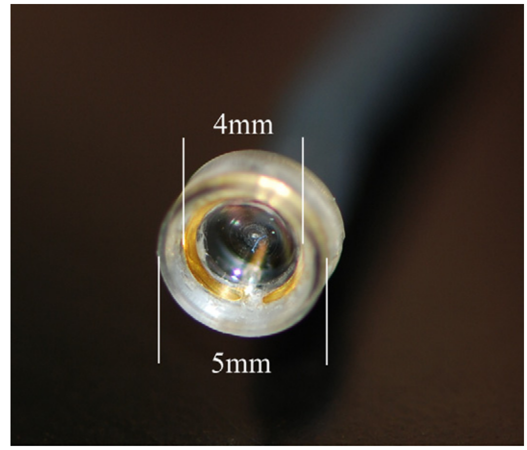

Fig. 1. Contact lens electrode used in the study seen from the concave side. The electrode is made of PMMA; a gold wire is embedded almost 360° circularly and polished to ensure smooth finish and good contact with the corneal surface.

2.3. Data analysis

Data extraction and analysis was performed using previously described software (Messias et al., 2008a). Briefly, the software semi-automatically reads out amplitudes and implicit times of a- and b-waves. B-wave analysis was performed after extracting oscillatory potentials using a fast Fourier transform (FFT) algorithm with a low and high cut-off of 75–300 Hz. For low intensity flashes an estimate of rod sensitivity was performed using a Naka–Rushton fit (Formula (1)) (Naka and Rushton, 1966). ERG responses of 11 steps (0.000003–0.3 scot cd s/m^2) were related to flash intensity (scot cd s/m^2) and parameters of the Naka–Rushton fit (V_{max} as maximum response; k as intensity needed for 50% of V_{max}; n as gradient of fit) were extracted (Formula (1)).

$$V(I) = \frac{V_{max} \times I^n}{I^n + k^n} \quad (1)$$

Additionally, a-wave and b-wave amplitudes and implicit times of high luminance flashes (1–60 scot cd s/ms) of a mixed rod-cone response were analyzed for both types of electrodes. Oscillatory potentials (OP) were analyzed after offline band-pass filtering with 75–300 Hz of responses elicited by intense flash stimuli (1–60 scot cd s/m^2). OPs were automatically calculated as area-under-the-curve (AUC) by the software between the a-wave and the peak of the b-wave.

2.4. Electrodes

The CLE of our laboratory has been previously described (Thaler et al., 2008). In short, a custom made PMMA contact lens with a diameter of 5 mm serves as base for an embedded circular gold wire. The contact lens possesses a concave impression to fit the average rat cornea; the opposite part is planar (Fig. 1). The gold wire is inserted from the planar side via a hole through the PMMA corpus of the CLE to the concave side where it is embedded in a pre-lathed circular groove. Finally the components (gold wire and PMMA) were glued and the gold wire was milled to guarantee a smooth finish with the PMMA material (Fig. 1).

The body of the new electrode consists of a hollow cylinder of PMMA with an internal diameter of 8 mm and an outside diameter of 10 mm which was constructed to expose the cornea and the adjacent conjunctiva. Fitting the cylinder over the animal's eye pushes the eyelids backwards such that the potential of the DTL-electrodes to contact with surfaces other than the cornea is minimized. The opening of the cylinder which faces the cornea contains drill holes