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Improving the quality of electroretinogram recordings using active electrodes

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ABSTRACT

The aim of this study was to compare the quality of electroretinogram (ERG) recordings using a custom built active electrode with attached amplifier versus a standard (passive) ERG electrode. Scotopic and photopic ERG responses were recorded from five adult albino rabbits using a custom built active electrode on one eye and a passive electrode on the other. For the active electrode, the ERG-jet electrode (Universo S.A., La Chaux-De-Fonds, Switzerland) was used as the transducer with the cable cut short and soldered directly to the input of a customized amplifier. The passive electrode was a standard ERG jet electrode. The signal to noise ratio and reproducibility of ERGs were compared. The noise was significantly lower in the active electrode compared to the passive electrode ($p = 0.009$) resulting in signals being recorded at lower stimulation strengths with the active electrode. The scotopic a-wave was significantly larger in the active electrode at all supra-threshold stimulation intensities ($p < 0.05$) and the scotopic b-wave amplitudes were also higher in the active electrode at all supra-threshold stimulation intensities but was only statistically significant between −3.25 and −1 log cd.s.m^{-2} ($p < 0.05$). The photopic a- and b-wave amplitudes were also higher in the active electrode and statistically significant between −0.75 and 0.48 log cd.s.m^{-2} for the a-wave and −1.25 to −1 log cd.s.m^{-2} for the b-wave ($p < 0.05$). The intra-observer repeatability, inter-sessions reproducibility and reliability of the signals were better in the active electrode as evidenced by lower coefficient of variation (CV) and coefficient of repeatability (CR) with high intra-class correlation coefficient (ICC) of the a- and b-wave parameters of the active electrode. These findings suggest that the custom built active ERG electrode produces less noise than the passive electrode, allowing responses to be recorded at lower stimulation strengths. It produces greater signal amplitudes and improved reproducibility and is therefore a better device for investigating retinal function.

1. Introduction

Line noise refers to the electrical interference induced in the cable that connects an electrode to the amplifier. Since the input impedance of an amplifier is high, noise can be generated in the cable by capacitive or magnetic coupling from the environment. These effects can be reduced, to some extent, by shielded or twisted-pair cables. The main type of noise interference comes from 50 Hz generated in the power mains lines and electrical sockets. This lies within the bandwidth of the electroretinogram (ERG) signal (1–300 Hz) and therefore strongly interferes with the quality of the recorded signal.

Many electrodiagnostic recording systems use active electrodes to overcome line noise. This involves coupling the first amplifier (often referred to as a pre-amplifier) as close as possible to the recording electrode. The electrical noise is reduced by impedance transformation since the low output impedance of the amplifier is almost impervious to electrical or magnetic interference. Active electrodes have been successfully implemented in several recording electrodes including electroencephalogram (EEG) (Taheri et al., 1994; Fonseca et al., 2007), electrocardiogram (ECG) (Bergey et al., 1971; Betts and Brown, 1976; Lopez and Richardson, 1969) and electromyography (EMG) (Potter and Menke, 1970; De Luca et al., 1979; Nishimura et al., 1992) but, to our knowledge, never previously been used in an ERG electrode. It was therefore the aim of this study to develop an active ERG electrode using a low noise buffer amplifier and to compare the quality of the recordings with a standard passive ERG electrode.

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2. Materials and methods

2.1. Animals

The experimental protocol in this study was conducted following approval from the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong. The study complies with the Guidelines of the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research. Five healthy adult New Zealand albino rabbits were provided by the Laboratory Animal Service Center of the Chinese University of Hong Kong. The rabbits were housed in a condition with a temperature maintaining at 19 ± 3 °C in relative humidity of 55 ± 10% and a 12-h:12-h dark:light cycle with clean standard diet and water ad libitum.

2.2. Active electrode and passive electrode

The active electrode was constructed by cutting the cable of an ERG-jet electrode (Universo S.A., La Chaux-De-Fonds, Switzerland) short (approx. 10 mm length) and soldering directly to the input of an operational amplifier (Analog Devices ADA-4899). The amplifier was configured as a unity gain buffer amplifier and powered using two standard 9 V batteries. All electronic components were hermetically sealed in epoxy. The passive electrode was a standard ERG-jet electrode (Universo S.A., La Chaux-De-Fonds, Switzerland) which is supplied with a cable of approximately 1 m in length.

2.3. Recording setup

The full-field flash ERG was recorded using an Espion E3 system (Diagnosys LLC, Littleton, MA). Five adult albino rabbits were dark-adapted for 20 min before being anaesthetized under dim red light by intramuscular injection of ketamine (35 mg/kg), xylazine (5 mg/kg) and aceptomazine (1 mg/kg). One drop of topical anesthesia was applied in each eye. The depth of anesthesia was verified by monitoring the palpebral or pinna reflex. The pupils were fully dilated by applying 0.5% tropicamide and 0.5% phenylephrine hydrochloride eye drops before recording. The active electrode was placed on one eye and a passive electrode on the fellow eye. For each recording session the eye chosen for the active electrode was alternated to ensure there was no bias. Hypromellose eye gel 0.3% was applied to prevent dry eye. The skin near the outer canthus was shaved and the reference electrode applied to the skin using Ten20 conductive gel (Weaver and Company, CO, USA). A gold wire was used as the ground electrode and this was inserted subcutaneously near the ear. After the completion of scotopic stimulation, the animals were light-adapted for 10 min before performing the photopic run stimulations.

Initially, the electrical recordings were taken without any stimulus in order to measure the background noise levels. For the scotopic flash stimulations between ten to forty responses were averaged depending on flash strength with stimuli ranging from −3.75 to 1.00 log cd.s.m^{-2}. For photopic responses, nine different stimulus strengths between −1.50 and 0.48 log cd.s.m^{-2} were used with a rod saturating background light at 30 cd s.m^{-2}. The inter-stimulus interval between flashes varied from 5s at the lowest stimulus strengths to 20 s at the highest stimulation strength.

During the recording the animals were monitored with a FLIR ONE thermal imaging camera (FLIR Systems Inc., USA) to ensure the electrical components did not cause excessive overheating of the ocular or adnexal tissues. A thermal image was taken at the end of each recording sessions for documenting the maximum temperatures reached by the electrical components and the tissues near the electrode.

2.4. Signal analysis

All ERG waveforms were analyzed using a customised Matlab (MathWorks, Natick, MA) program. The signals were filtered using a butterworth second order bandpass filter from 1 to 300 Hz and the signal epochs averaged. In compliance with the International Society for Clinical Electrophysiology (ISCEV) guidelines, the a-wave amplitude was measured from baseline to the maximum negative trough and the b-wave amplitude was measured from the a-wave trough to the maximum positive peak (Marmor et al., 2009). For the noise measurement, root mean square (RMS) of noise amplitude was measured.

2.5. Statistics

Data was analyzed with SPSS (Version 22.0.0, IBM®). Mann-Whitney *U* test was used for comparing the mean a- and b-wave amplitude values in all ERG responses and mean noise level between active and passive electrodes. For all analyses, a 2-sided $P < 0.05$ was considered statistically significant.

The coefficient of variation (CV) was calculated as the within–subject standard deviation (Sw) divided by the overall mean. The coefficient of repeatability (CR) was defined by $1.96 \times \sqrt{2}$ x Sw, which is equal to 2.77 Sw. The intra-class correlation coefficient (ICC) was calculated with a one-way random effects model. The ICC values were classified as follows: > 0.75 was excellent, 0.40–0.75 was fair to good and < 0.40 was poor (Fleiss, 1986).

3. Results

3.1. Background noise

The mean ± standard deviation (SD) root mean square (RMS) of the active and passive electrodes were 1.557 ± 0.150 µV (range: 1.411–1.798 µV) and 3.569 ± 0.828 µV (range: 2.651–4.443 µV), respectively. The noise was significantly lower in active electrode compared with passive electrode (p = 0.009). A Fast Fourier Transform (FFT) showed no 50 Hz component in the active electrode which was evident in the passive electrode (Fig. 1). The contribution of 50 Hz in the passive electrode was variable from one recording session to another. However, the active electrode did not show any significant 50 Hz component in any of the recording sessions.

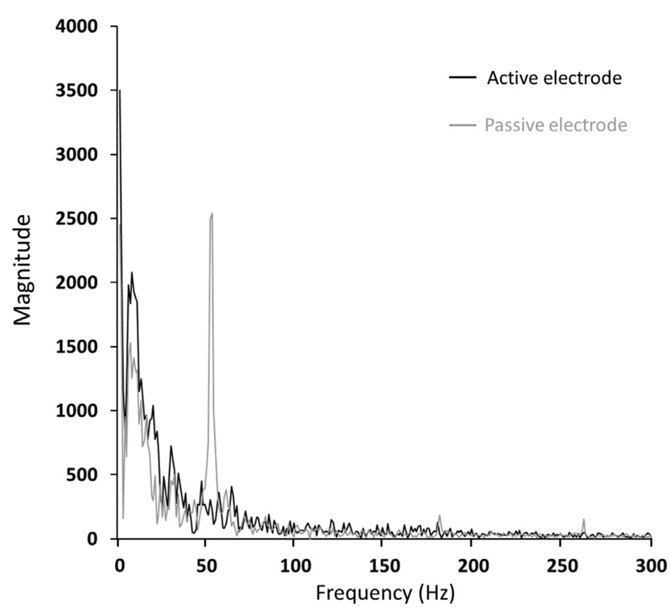

Fig. 1. Fast fourier transform (FFT) from a typical recording. There is a significant 50 Hz contribution in the passive electrode which is not evident in the active electrode design.