Full-field electroretinogram recording in the Göttingen minipigs

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Introduction

Guidelines for electroretinography examinations for humans have been published and are regularly updated by the International Society for Clinical Electrophysiology of Vision (ISCEV). The aim of these guidelines is to propose standardized and reproducible protocols for electroretinographic examination. In animals, particular attention should be paid to the anatomical/physiological specificities of the tested species (*e.g.*, diurnal or nocturnal) when determining which procedure to use. The Société Française d'Etudes et de Recherches en Ophtalmologie Vétérinaire (SFEROV) proposes an electroretinogram (ERG) recording procedure based on simultaneous binocular stimulation in light-adapted dogs taking into consideration the above parameters. As the minipig is a diurnal species, the purpose of this study was to adapt and evaluate the dog protocol for use in minipigs.

Materials and methods

Animals

A total of 162 Göttingen minipigs (81 females and 81 males), aged 4-6 months, were examined in this study.

Recording equipment

The electroretinographic examination was performed simultaneously on both eyes with the use of a bilateral system of stimulation and recording (Visiosystem electroretinograph). Two sclerocorneal clip electrodes, placed symmetrically on the scleral conjunctiva of both eyes, were used as active electrodes. Needle electrodes were used as reference and ground electrodes. All the electrodes were connected to an amplifier, which was connected to the Visiosystem electroretinograph. The Visiosystem was used to generate the flash stimuli: bright white light created by a xenon capacitive discharge photostimulator with an intensity of 2.5 cd.s/m^2 and a duration of 20 µs. Filters [neutral density and blue Kodak Wratten 98 (440 nm)] were inserted between the lights and the eyes in order to change the flash stimulus characteristics (intensity and spectral composition): dim blue light with an intensity of 0.025 cd.s/m^2. The flash lamps were symmetrically positioned at 2 cm from each eye in order to obtain full field conditions for the retinal stimulation.

Recording method

Prior to the electroretinogram recordings, the pupils were dilated with 1% tropicamide (Mydriaticum™). After a 2-hour pre-adaptation period in a photopic environment (30 cd/m^2), the animals were anesthetised by an intramuscular injection of medetomidine (Domitor™) in combination with tiletamine and zolazepam (Zoletil™). The head restraint system and the electrodes were positioned. The cornea was kept hydrated with drops of carbopol gel (Ocrygel™).

The first recording was performed in a photopic environment with high-intensity stimulation, to extract the cone-dominated response. The flicker response was recorded immediately afterwards. The minipigs were then subjected to dark adaptation for 20 minutes. During this period, the retina was stimulated every 4 min *(i.e.* at 4, 8, 12, 16 and 20 minutes) to extract the rod-dominant responses. Lastly, the response to a single white flash was recorded to obtain the maximal combined rod-cone response.

Data analysis

The a-wave and b-wave peak times and amplitudes were measured and statistically analyzed using SAS software (SAS Release 8.02, SAS Institute Inc). For each of the statistical comparisons, normality and homogeneity of variances were evaluated.

Results

Representative electroretinogram recordings taken from one of the 162 minipigs examined in this study are illustrated in Figure 1.

The results of the statistical analysis are presented in Table 1 (photopic electroretinogram), Table 2 (scotopic electroretinogram) and Table 3 (single-flash electroretinogram). The data collected from the right and left eyes of the same animal were averaged. No significant gender differences were observed, except for higher b-wave amplitude for the photopic electroretinogram responses in females when compared to males (48.14 µV±12.91 vs. 42.88 µV±10.67; p=0.005; Table 1).

Discussion

Recording method

Photopic and scotopic electroretinogram were successfully recorded from anaesthetized minipigs using sclerocorneal clip electrodes. This type of electrode allows the animal's head and ocular globes to be maintained in a stable position during the entire examination and avoid a direct contact with the cornea. Electroretinogram recordings were performed simultaneously in both eyes which ensured same environmental and anaesthetic conditions. Furthermore, this method allows the examination time to be shortened and avoided gaseous anesthesia, such as halothane inhalation anaesthesia, which is known for its negative effect on electroretinogram amplitude[1].

There was no gender difference, except for the photopic b-wave amplitude which was statistically higher in females than in males comparable to observations made in humans.

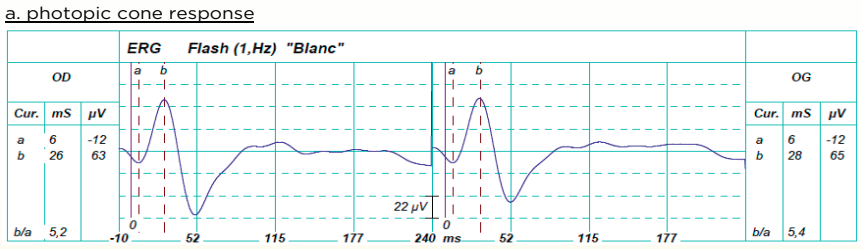
High coefficients of variation were noted for the amplitude values of almost all recordings. Different variables could explain the differences: absence of mydriasis measurement, variation in the degree of fundus pigmentation, variation of the head position, small differences in stimulator positioning affecting the amount of delivered light. The slightly exponential aspect of the increase in peak-time and amplitude values during the dark-adaptation period suggested that retina adaptation state would be achieved after 30 min of darkness, as shown in previous studies using other species of animal. It was decided to forgo monitoring of the electroretinogram recordings beyond the 20-min period, as 20 min appeared to be an acceptable compromise between the anesthetic method used and the length of the examination. The photopic electroretinogram was recorded prior to the scotopic electroretinogram to facilitate the positioning of the electrodes on the minipigs during the light-adaptation period and to shorten the delay before the examination.

The single flash, recorded after 20-min dark adaptation, was high, with values higher than those obtained during the photopic recording and the recording at the conclusion of the 20-minute dark-adaptation period. This confirmed that the single-flash electroretinogram had recorded general rod and cone responses.

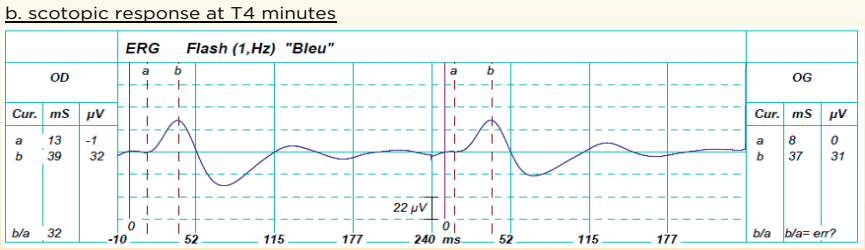
Electroretinogram recordings

Figure 1. Examples of ERG recordings from one minipig

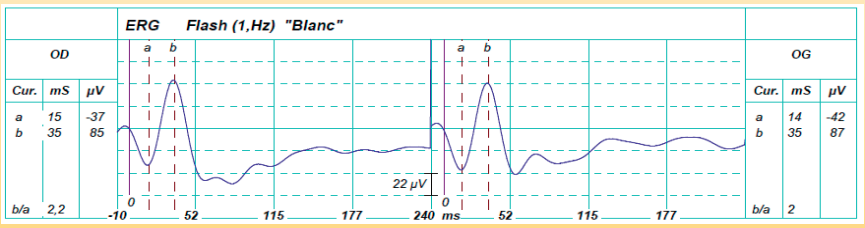
a. photopic cone response



b. scotopic response at T4 minutes



c. maximal combined rod-cone response (single flash)



The pictures show the photopic cone response (a), the scotopic response after 4 minutes of dark adaptation (b) and the maximal combined rod-cone response (c).

Table 1: Statistical results for a- and b-wave amplitude and peak time for photopic ERG

Label	Sex	N	Mean	SD	CV	p-Value
1. a-wave amplitude and peak time for photopic ERG						
a-wave amplitude	Female	81	8.25	3.67	44.47	0.06882
	Male	81	7.19	3.25	45.24	
a-wave peak time	Female	81	8.44	1.19	14.07	0.62654
	Male	81	8.37	1.22	14.56	
2.b-wave amplitude and peak time for photopic ERG						
b-wave amplitude	Female	81	48.14	12.91	26.81	0.00530
	Male	81	42.88	10.67	24.87	
b-wave peak time	Female	81	30.96	1.14	3.67	0.41354
	Male	81	31.07	1.25	4.02	

Standard deviation (SD), Coefficient of variation (CV)

Table 2: ERG Statistical results for a- and b-wave amplitude and peak time for scotopic ERG

Dark adaptation	Sex	Label	N	Mean	SD	CV	p- value
1. b-wave amplitude for scotopic ERG							
T 0 min	Female	b-wave amplitude	81	31.86	12.93	40.59	0.8564
	Male	b-wave amplitude	81	31.73	12.88	40.60	
T 4 min	Female	b-wave amplitude	81	53.05	16.80	31.66	0.7177
	Male	b-wave amplitude	81	52.06	17.89	34.37	
T 8 min	Female	b-wave amplitude	81	63.77	21.31	33.42	0.6973
	Male	b-wave amplitude	81	62.51	20.00	32.00	
T 12 min	Female	b-wave amplitude	81	70.60	19.95	28.26	0.3560
	Male	b-wave amplitude	81	67.61	21.11	31.22	
T 16 min	Female	b-wave amplitude	81	74.13	20.79	28.04	0.5495
	Male	b-wave amplitude	81	72.09	22.41	31.08	
T 20 min	Female	b-wave amplitude	81	77.15	19.25	24.95	0.4965
	Male	b-wave amplitude	81	74.57	21.61	28.97	
2. b-wave peak time for photopic ERG							
T 0 min	Female	b-wave peak time	81	39.44	39.50	1.93	4,91	0.6596
	Male	b-wave peak time	81	39.44	39.25	1.74	4.41	
T 4 min	Female	b-wave peak time	81	42.49	42.50	2.23	5.25	0.8998
	Male	b-wave peak time	81	42.78	42.50	2.20	5.14	
T 8 min	Female	b-wave peak time	81	44.09	44.00	2.63	5.97	0.1391
	Male	b-wave peak time	81	44.81	45.00	2.62	5.85	
T 12 min	Female	b-wave peak time	81	45.40	45.50	2.64	5.81	0.2739
	Male	b-wave peak time	81	45.93	46.00	2.83	6.17	
T 16 min	Female	b-wave peak time	81	46.86	46.50	2.55	5.44	0.9920
	Male	b-wave peak time	81	47.03	47.00	2.91	6.19	
T 20 min	Female	b-wave peak time	81	47.27	47.00	2.17	4.58	0.8601
	Male	b-wave peak time	81	47.42	47.50	2.73	5.76	

Standard deviation (SD), Coefficient of variation (CV)

Table 3: Statistical results for a- and b-wave amplitude and peak time for single flash ERG

Label	Sex	N	Mean	SD	CV	p-Value
1. a-wave amplitude and peak time for single flash ERG						
a-wave amplitude	Female	81	29.98	15.31	51.08	0.2310
	Male	81	26.32	10.21	38.78	
a-wave peak time	Female	81	16.73	1.74	10.38	0.5995
	Male	81	16.57	1.57	9.49	
2. b-wave amplitude and peak time for single flash ERG						
b-wave amplitude	Female	81	115.97	22.94	19.78	0.1154
	Male	81	110.23	18.16	16.48	
b-wave peak time	Female	81	41.32	1.92	4.64	0.2471
	Male	81	40.95	1.62	3.96	

Standard deviation (SD), Coefficient of variation (CV)

Conclusion

In the present study we have adapted the SFEROV electroretinogram recording procedure to the Göttingen minipigs. We presented reference data obtained from 162 animals. Our method is appropriate for use in routine GLP toxicology studies with reliable results under photopic or scotopic conditions.

Reference

1. Acland GM, Forte S, Aguire GD. Halothane effects on the canine electroretinogram. 12th Annual Meeting of the American College of Veterinary Ophthalmologists 1981, 66-83.