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## Steady-state electroretinograms and pattern electroretinograms in pigs

Received: 6 April 2000  
Revised: 6 June 2000  
Accepted: 20 June 2000

**Abstract** *Background:* Electroretinograms (ERG) or pattern-electroretinograms (PERG) could be valuable for the quantification of potential damage to the pig retina by experimental erbium:YAG laser treatment. We therefore performed a normative study of ERGs and PERGs in pigs. *Methods:* We recorded ERGs and PERGs under general anaesthesia in two experiments. In experiment 1 we examined eight eyes from six pigs of 20–25 kg body weight; in experiment 2 we examined four eyes from four pigs of 40–45 kg body weight. We used flash and checkerboard stimuli. In experiment 1, the stimulus parameters were mean luminance 48.3 cd/m<sup>2</sup> for checkerboard stimuli, 96.6 cd/m<sup>2</sup> for ERG, check sizes of 4°, 8°, and 16°, temporal frequencies were 16 Hz for ERG and 8 rev/s for PERG. Three measurements were repeated after two weeks. Stimulus parameters for experiment 2 were luminance 175 (350) cd/m<sup>2</sup>, check sizes 1.6°, 3.2°, 6.7°, and 16°, temporal frequencies 6.3 Hz for ERG and 8 rev/s for PERG. Recordings were subjected to Fourier analysis. *Results:* In experiment 1 the mean ERG amplitude was 1.02±0.89 μV

with a coefficient of variation of 42% for repeat sessions. The mean PERG amplitudes were 0.53±0.25 μV for 16° checks, 0.36±0.21 μV for 8°, and 0.25±0.17 μV for 4°. The mean coefficient of variation between two measurements was 103% for 16° checks, 24% for 8°, and 116% for 4°. In experiment 2 the mean ERG amplitude was 9.72±3.96 μV. The mean PERG amplitudes were 0.77±0.50 μV for 16° checks, 0.09±0.16 μV for 6.7°, 0.07±0.13 μV for 3.2°, and 0.08±0.09 μV for 1.6°.

*Conclusions:* It was possible to record ERGs and PERGs in pigs. However, the ERG amplitudes were small; PERG amplitudes were even smaller in both groups and cannot be reliably recorded. A problem for both ERG and PERG was the high intra-individual and interindividual variability. Therefore, only very extensive damage to the retina by vitrectomy or Er:YAG laser treatment might lead to a significant change in the ERG or PERG amplitudes.

**Keywords** Electroretinogram · Pattern electroretinogram · Pig · Vitreous surgery · Normative data

This paper was presented in part at the DOG meeting in Berlin, 1999

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### Introduction

Proliferative vitreo-retinopathy remains a challenge in the treatment of retinal detachment. Removal of the associated epiretinal membranes is often impossible or

prone to retinal-hole formation. A promising solution to this problem is membrane removal by erbium:YAG (Er:YAG) laser [8, 21]. To develop this technique we use a pig model, since the structure of the pig eye has been shown to be similar to that of the human eye (e.g. [9]).

Electrophysiological methods may be useful for assessing any functional damage caused by laser treatment. The pattern electroretinogram (PERG) reflects ganglion cell activity [1, 3–5, 10, 12, 16, 24], and the flash electroretinogram (ERG) is generated by receptors and bipolar cells. Damage to the superficial nerve fibre layer thus should affect the PERG, whereas damage to the outer retinal layers caused by laser-induced acoustic pressure waves [7] should affect the ERG. We report here on our attempts to record reliable ERG and PERG signals in our pig model before surgical intervention. Rapid stimulation (steady-state) was applied, since Fourier-analytic methods allow amplitudes and significance values of small electrophysiological responses to be objectively measured.

After our initial series of animals (experiment 1) we were disappointed with the small and unreliable signals. As possible reasons for this we considered the age of the animals (too young), the temporal frequency of the stimulation (too high) and stimulus luminance (increasing luminance typically increases amplitude [2]).

Thus a second series (experiment 2) was run with older animals, and a number of stimulus parameter changes that should have increased the electroretinographic signal strength, again with disappointing results.

## Materials and methods

### Animals

We performed ERG and PERG measurements in pigs (Deutsche Landrasse). The animals were treated according to the ARVO statement for the use of animals in ophthalmic and vision research. Consent of the local authorities was given (Regierungspräsidium Freiburg).

*Experiment 1* In six animals with a body weight of 20–25 kg we recorded from eight eyes during miosis (in four pigs only one eye each, in two both eyes sequentially).

*Experiment 2* In four animals with a body weight of 45–50 kg we recorded from four eyes during mydriasis after tropicamide application.

The pigs were denied food 24 h before an operation but had access to water. Premedication consisted of 0.2 mg flunitrazepam/kg body weight, and 7 mg ketamine/kg, both given intramuscularly. All measurements were done under general anaesthesia: After pre-oxygenation, general anaesthesia was started with 4 mg propofol i.v./kg body weight. Muscle relaxation was achieved by 0.2 mg vecuronium/kg body weight. Pigs were intubated with a low-pressure cuff tube (6–7 mmHg cuff pressure). Intermittent positive pressure ventilation (frequency 12/min, volume 10 ml/kg body weight) was applied. Anaesthesia was maintained using oxygen/nitrous oxide=0.4, and 7 mg·kg<sup>-1</sup>·h<sup>-1</sup> propofol, 0.015 mg·kg<sup>-1</sup>·h<sup>-1</sup> fentanyl, 0.4 mg·kg<sup>-1</sup>·h<sup>-1</sup> vecuronium, and 10 ml·kg<sup>-1</sup>·h<sup>-1</sup> Ringer's solution intravenously. Monitoring comprised electrocardiography and pulse oximetry. Anaesthesia was kept at medium levels; animals responded to loud noise with slow eye movements and increased muscle tonus.

We performed retinoscopy in four of six eyes in experiment 1 and in all of the animals in experiment 2. The refractive error was low enough for corrective glasses to be dispensable.

### Stimulation

Stimuli were flashes (white screen, 15 ms duration) to evoke the ERG and phase-reversing checkerboards (98% contrast) to evoke the PERG. They were presented on a cathode ray tube (CRT; GD403, Richardson Electronic) with a stimulus field size of 32°×27°, viewed from a distance of 57 cm. The CRT was aligned to the eye by observing the corneal reflex evoked by the checkerboard. For PERG recording, checkerboards with different check sizes were used. All experiments were performed under photopic conditions, set by the stimulus luminance (see below) and ambient room lighting of 13 lux.

Experiments 1 and 2 differed in luminance, check size, and reversal frequency. In experiment 1 the mean luminance of the monitor was 48.3 cd/m<sup>2</sup> for checkerboard stimuli (evoking the PERG) and 96.6 cd/m<sup>2</sup> for ERG. Check sizes were 4°, 8°, and 16°. Temporal properties were 16 Hz for ERG and 8 rev/s for PERG. In experiment 2 the mean luminance of the monitor was higher than in experiment 1: 175 cd/m<sup>2</sup> for checkerboard stimuli (evoking the PERG) and 350 cd/m<sup>2</sup> for ERG. Check sizes were 1.6°, 3.2°, 6.7°, and 16°. Temporal properties were 6.3 Hz for the flash ERG and 8 rev/s for PERG.

### Recording and analysis

Signals were recorded with DTL electrodes. These were placed in the lower limbus and their ends were taped with adhesive strips, keeping them insulated from the skin. A gold cup reference electrode was placed on the forehead. A ground electrode was attached to the earlobe. The electrical impedance for all electrodes was less than 3 kΩ. Signals were amplified, filtered (0–40 Hz) and digitised. Automatic artifact suppression was used with a threshold of ±100 μV. The 48–240 sweeps were averaged in a blocked design across conditions.

To quantify our results, we performed a Fourier analysis. Response amplitude was defined as spectral magnitude at the flash frequency (ERG) or at the reversal frequency (PERG). Statistical significance was calculated for each response based on a signal-to-noise algorithm [14]. Only those responses where the amplitude for 16° check sizes was significant ( $P > 0.05$ ) were taken for further analysis.

Three animals in experiment 1 were examined twice, with an interval of 15 days between the two sessions. To quantify the reproducibility, we calculated the coefficient of variation (CV) for each animal by calculating the mean ( $m$ ) and standard deviation (SD) of the two sessions:

$$CV = \frac{m}{SD} \times 100\%$$

These values were averaged across animals.

## Results

### Experiment 1

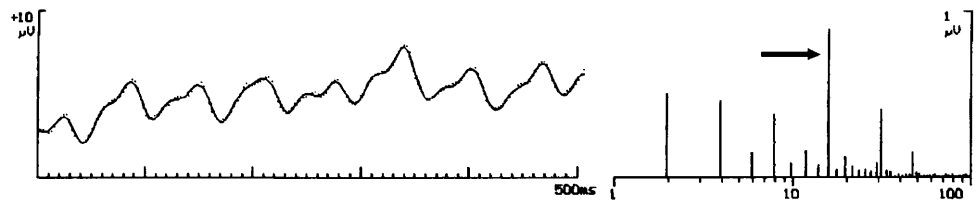
Significant ( $P < 0.05$ ) ERGs and PERGs could be measured in eight eyes from six pigs. Table 1 gives the details of our findings. Fig. 1 shows a typical steady-state ERG with its Fourier spectrum. In all animals well-defined ERG responses were found. The mean amplitude was  $1.02 \pm 0.89 \mu\text{V}$  (range 0.23–3.11 μV). The mean coefficient of variation for repeat sessions was 62.6%.

The amplitudes of the steady-state PERGs were too small to be recognised in the original traces. Only after

**Table 1** ERG and PERG amplitudes in experiment 1. In three animals the experiments were repeated after 2 weeks (1st/2nd session), allowing us to assess reproducibility. The ERG amplitudes were reasonably large, whereas the PERG amplitudes were very small. The standard variation was large. *OD* right eye, *OS* left eye

Animal	1st Session				2nd Session			
	Flash ERG	Pattern ERG			Flash ERG	Pattern ERG		
		16°	8°	4°		16°	8°	4°
021 OD	0.884	0.192	0.091	0.168	2.11	0.405	0.384	0.289
022 OD	0.233	0.41	0.266	0	1.98	0.378	0	0
023 OD	3.11	0.886	0.408	0.381	2.4	0.288	0	0
026 OD	0.938	0.889	0.766	0.517				
026 OS	0.615	0.352	0.175	0.101				
027 OD	0.727	0.444	0.46	0.398				
027 OS	1.11	0.636	0.393	0.225				
028 OS	0.55	0.429	0.291	0.188				
Mean	1.02	0.53	0.36	0.25				
SD	0.89	0.25	0.21	0.17				

**Fig. 1** A steady-state electroretinogram and its Fourier spectrum from an animal in experiment 1. The ERG trace (left) shows well-defined peaks, giving rise to the 6.3 Hz peak in the Fourier spectrum (right, arrow)



Fourier analysis was a response at the reversal rate detected. The mean amplitudes were  $0.53 \pm 0.25 \mu\text{V}$  for  $16^\circ$  check sizes,  $0.36 \pm 0.21 \mu\text{V}$  for  $8^\circ$  check sizes, and  $0.25 \pm 0.17 \mu\text{V}$  for  $4^\circ$  checks. The mean coefficient of variation for repeat sessions was 42.7% for  $16^\circ$  checks, 123.3% for  $8^\circ$  checks, and not calculable due to too few measurements for  $4^\circ$  checks.

## Experiment 2

Significant ( $P < 0.05$ ) ERGs and PERGs could be measured in four eyes from four pigs. Table 2 gives an overview of our findings. In all animals well-defined ERG responses could be observed. The mean amplitude was  $9.72 \pm 3.96 \mu\text{V}$  (range 5.67–14.6  $\mu\text{V}$ ).

Similar to the findings in experiment 1, the small amplitudes of the steady-state PERGs were too small to be recognised in the original traces. Only after Fourier analysis was a response at the reversal rate detected. The mean amplitudes were  $0.77 \pm 0.50 \mu\text{V}$  for  $16^\circ$  checks,  $0.09 \pm 0.16 \mu\text{V}$  for  $6.7^\circ$  checks,  $0.07 \pm 0.13 \mu\text{V}$  for  $3.2^\circ$  checks, and  $0.08 \pm 0.09 \mu\text{V}$  for  $1.6^\circ$  checks.

## Comparison between experiment 1 and experiment 2

In experiment 2 we obtained generally higher amplitudes than in experiment 1. The amplitude of the ERG was 9.5 times higher, the amplitude of the PERG for  $16^\circ$  checks was 1.5 times higher. Consequently, the variability

**Table 2** ERG and PERG amplitudes in experiment 2, which was designed to yield higher amplitudes than experiment 1. While the ERG amplitudes were 9.5 times larger, the PERG amplitudes and the standard deviation were comparable

Animal	Flash ERG	Pattern ERG			
		16°	6.7°	3.2°	1.6°
032	14.6	1.32	0.277	0.221	0.189
031	7.52	0.379	0	0	0.019
030	5.67	0.319	0	0	0.041
029	11.1	1.08			
Mean	9.73	0.77	0.09	0.07	0.08
SD	3.96	0.50	0.16	0.13	0.09

was lower in experiment 2. For quantification, we calculated the coefficient of variation between animals for each experiment:

$$- CV_i = \frac{m_i}{SD_i}, \text{ with } m_i = \text{mean, of experiment } i (i = 1 \text{ or } 2) \\ \text{across animals, } SD_i = \text{SD across animals for experiment } i.$$

$CV_1$  was 86.8% for the ERG, and 47.6% for the PERG;  $CV_2$  was 40.7% for the ERG, and 64.8% for the PERG ( $16^\circ$  checks).

## Discussion

Flash and pattern stimuli evoked electrophysiological responses (ERG and PERG) from pig retina. However, the

initial results provided disappointingly low amplitudes, prompting us to perform a second series of experiments. For experiment 2 we altered stimulation parameters (higher luminance, lower temporal frequency, more appropriate check sizes) and animal age with the aim of evoking larger electrophysiological responses. While amplitudes were indeed larger, the variability remained at around 50%.

Published reports on electrophysiological recordings in pig retina are very scarce. Only transient flash ERG in pigs has been reported [11, 18, 22]. Therefore, our steady-state ERG recordings can only be compared to findings in other species. In experiment 1 the ERG and PERG amplitudes were about a quarter of the amplitude in monkeys [13] or humans [15]. The PERG amplitude in pigeons and the little owl [17, 19] was about the size of the PERG in pigs. Yin et al. [23] published PERGs for cats but did not report quantitative amplitude data; the article, however, hints at difficulties in measuring the small amplitudes. The best results so far have been presented by Rosolen et al. [18]. They recorded photopic transient ERGs with xenon flash stimulation of 2.5 cd·m<sup>-2</sup>·s. With careful procedures and high stimulation intensities they obtained recordings whose CV we calculated from their data to lie between 10% and 50%.

For the intended purpose of functional assessment, the variability of our recordings appears too high. What could be the reason for this large variability? Stimulation and recording parameters are unlikely candidates, since they were very similar to our routine set-up in patients, where we found reproducibility within 10% [15]. The situation differs for ERG and PERG: The ERG has generally a much higher amplitude than the PERG, and our PERG amplitudes were close to noise, suggesting that PERG variability is partly due to the low signal-to-noise ratio. For the ERG, we hypothesise that (1) varying depth of the anaesthesia and (2) young age of the animals explain most of the variability:

1. The depth of the anaesthesia changed from animal to animal and was difficult to monitor objectively and to maintain constant. This hypothesis appears likely, as Rosolen [18] found greater reproducibility in fully awake micropigs. Further, anaesthesia has been shown to affect ERG components [20].
2. We used very young pigs of nearly identical age. However, since postnatal development occurs at a different pace in each individual, maturation was not necessarily identical from animal to animal. Since maturation influences the ERG [6], the high interindividual variability might be due to a different stage of postnatal development in the animals. The fact that the variability of the ERG was larger in the group of younger animals supports this hypothesis.

It is generally accepted that a significant change between sessions has occurred if their results differ by more than two standard deviations. If we use this criterion it would be impossible to find out whether vitrectomy or laser therapy alters the PERG in young pigs because the variability of 50% which we found in our experiments is too high. ERG recordings could be used if we stimulate with a very bright monitor because there is a 40.7% variability. Therefore, destruction of more than 81.4% of the retina should significantly alter the ERG.

It is possible to obtain ERG and PERG recordings in young pigs, which are preferably used for experimentation as they are handled more easily than older animals. Unfortunately, both the ERG and the PERG suffer from large intra-individual and interindividual variability, so their use for comparing pre- and postoperative retinal function is limited. Only very bright stimuli for ERG recordings might allow some comparison between pre- and postoperative function of the retina. The use of older animals may be advantageous.

**Acknowledgement** We thank Dr med. vet. B. Eissner for the anaesthesia of our animals.

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