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The ERG of guinea pig (*Cavis porcellus*): comparison with I-type monkey and E-type rat

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Abstract

We have been in search of an alternate species for the monkey to study the effects of drugs on the I-type photopic electroretinogram (ERG) response that is typically seen in the cone-rich retina of the primate. The guinea pig has two types of cones, one of which contains a middle-wavelength sensitive pigment otherwise found only in Old World primates. We studied the Ganzfeld electroretinogram (ERG) of the guinea pig in relation to monkey and rat ERGs to learn whether the guinea pig might be a good animal model to study the 'primate-like' cone ERG. The guinea pig scotopic ERG was similar to other mammal ERGs and was not electronegative when fully dark-adapted. We saw no evidence of a negative-going scotopic threshold response (STR). The guinea pig photopic ERG a-wave is larger than that of the rat but much smaller than the primate a-wave, and it lacked a phasic d-wave. PDA eliminated guinea pig photopic a-wave and caused the OFF-response to long stimuli to invert polarity, as seen in monkey but not in rat. The guinea pig overall shows a weak I-type response and may be a useful substitute for primate in some studies of the photopic ERG.

Abbreviations: DBC – depolarizing bipolar cells; HBC – hyperpolarizing bipolar cells; HzC – horizontal cells; PDA – *cis*-2,3-piperidine-dicarboxylic acid.

Introduction

We have been in search of an alternate species for the monkey in which to study the effects of drugs on the I-type photopic electroretinogram (ERG) response that is typically seen in the cone-rich retina of the primate. The guinea pig (*Cavis porcellus*) has traditionally been regarded as a rodent, but recent phylogenetic analysis using amino acid sequence data indicates that the guinea pig has an ordinal status separate from *Rodentia* [1, 2] by an early evolutionary divergence [2]. Supporting evidence of this comes from retinal studies. Mammalian horizontal cells serve the rod and cone pathways separately: A- and B-type horizontal cell dendrites contact only cones, and B-type axon terminal systems contact only rods [3]. Rats and mice have only B-type horizontal cells, whereas the guinea pig has both axonless A-type and axon-bearing B-type horizontal cells [4]. This implies that the guinea pig has evolved a retinal organization for more complicated cone vision.

Like most other mammals, the guinea pig retina is rich in rods that contain typical mammalian rhodopsin with peak absorption (λ_{max}) of 497 nm [5, 6]. The early literature was inconsistent, however, about the presence of cones in the guinea pig retina. Granit [7] described the guinea pig as a purely rod animal. More recently, several cone types have been demonstrated in a number of mammalian species using cone-specific anti-visual pigment antibodies. In the guinea pig, two monoclonal antibodies specifically label 'blue-sensitive' and 'green- and red-sensitive' visual pigments of cones, thus providing evidence that the guinea pig retina contains at least two different

Table 1. Cone sensitivities in rat, mouse, guinea pig, and primate

	UV (nm)	S-Cone (nm)	M-Cone (nm)	L-Cone (nm)	Cones in retina (%)
Mouse, rat	360		510		1–3
Guinea pig		429	529		8–17
Macaque monkey		430	535	562	6–7
Human		430	531	561	5–7

*References. Cone proportions: mouse [11], rat [8], guinea pig [4], monkey [12], human [13]. Peak sensitivities of cone pigments: mouse and rat [14], guinea pig [6], monkey [15], human [14, 16].

types of cone photoreceptor pigments [8–10]. ERG flicker photometry studies indicated two classes of cones with peak sensitivities of 429 and 529 nm [6]. The presence of two classes of cones suggests a retinal basis for color discrimination, and behavioral tests have shown that guinea pigs have dichromatic color sensitivity with a spectral neutral point at about 480 nm [6].

The guinea pig is different from *Muridae*, such as rats and mice, which have an ultraviolet (UV)-cone with sensitivities at about 360 nm and a second cone type near 510 nm (Table 1). The guinea pig does not have a UV-cone, but it has an S-cone with the same spectral peak as the primate. Only Old World primates have cone pigments with peak sensitivity near 530–535 nm, similar to the 529-nm pigment of the guinea pig [14, 17]. Based on these similarities between the guinea pig and Old World primates including human, we explored the possibility that the guinea pig might be useful for some aspects of cone ERG research.

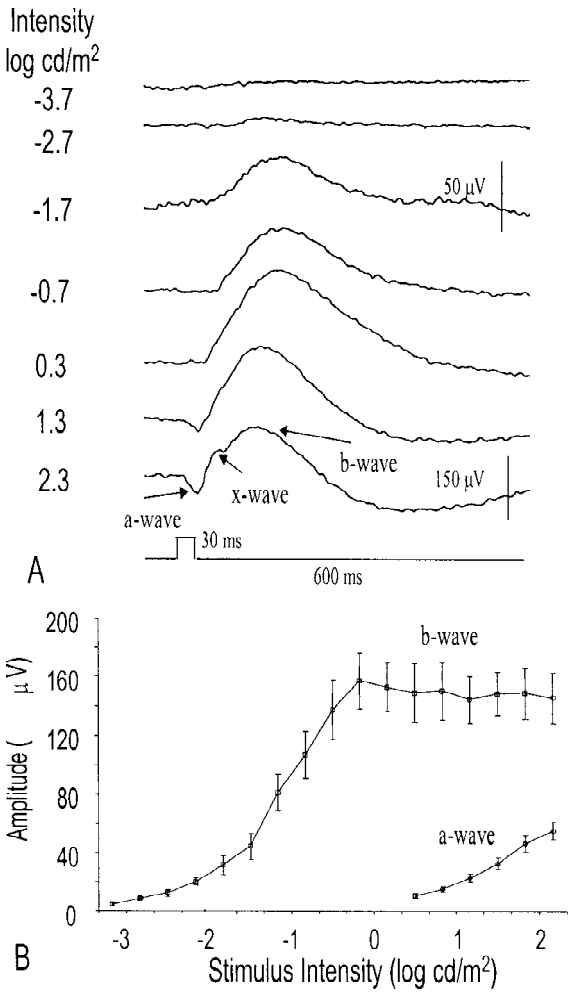

Figure 1. (A) Scotopic ERG of guinea pig to light flashes of increasing intensity. (B) V-Log I curve of these data. The averaged a-wave and b-wave values are from the right eyes of 12 guinea pigs. Bars show standard errors.

Materials and methods

These studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Protocols were approved by the University of Michigan. Twelve 500–600 g albino Dukin–Hartley guinea pigs were used. Animals were sedated with ketamine hydrochloride (20 mg/kg loading dose and 10 mg/kg per h maintenance i.m.) and xylazine (2 mg/kg loading dose and 1 mg/kg per h maintenance i.m.). ERGs were recorded from both eyes simultaneously using gold wire corneal electrodes after topical anesthesia (proparacaine 0.5%) and full pupillary dilation (phenylephrine HCl 10% and atropine 1%). A reference electrode was placed on the sclera 2 mm from the temporal limbus of each eye and the ground electrode was a subcutaneous stainless steel needle on the back. Signals were amplified at 10 000 gain between 0.1–1000 Hz (−3 dB points), and a 60-Hz notch filter minimized any power line noise. The responses were digitized at 1 kHz rate, averaged, stored, and analyzed off-line. The Ganzfeld stimulus (maximum 2.3 log cd/m^2) was reduced by neutral density filters. Thirty ms flashes were used for scotopic recordings after at least 1 h of dark adaptation.

Two rhesus monkeys (typical I-type ERG) and six Sprague–Dawley albino rats (typical E-type ERG) were included in this study using methods as previously described [18, 19]. From our measurements,

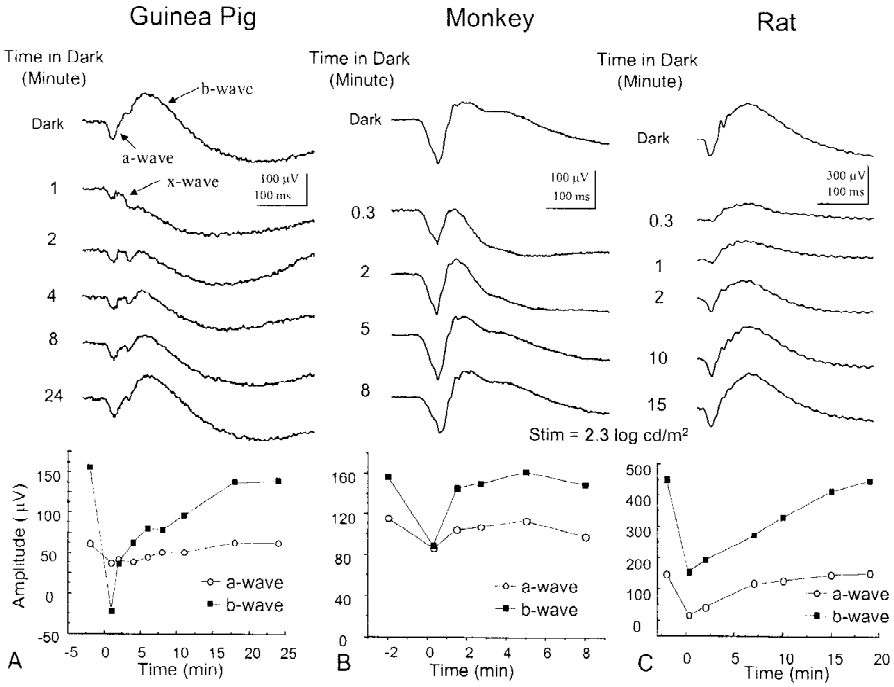

Figure 2. Recovery of dark-adapted ERG waveform after 1 min light exposure of 42 cd/m^2. Guinea pig shows an electronegative ERG when the retina is only partially dark-adapted. The fully dark-adapted guinea pig ERG is similar to that of monkey and rat, with the b-wave amplitude being larger than the a-wave. The low panels show the amplitude changes as a function of time. Only the guinea pig has a smaller b-wave than a-wave during the first few minutes of dark adaptation. Stimulus light intensity is 2.3 log cd/m^2.

the dilated pupil diameter of the adult guinea pig is 4 mm; the posterior nodal distance is approximately 6 mm. With these measurements and the schematic eye of rat and human primate [20], we calculated that for a fully dilated pupil under Ganzfeld brightness, retinal illuminance for guinea pig is similar to rat and monkey (1.0:1.3:1.4) and is within 0.15 log units. A 40 mM PDA (*cis*-2,3-piperidine-dicarboxylic acid, Sigma Chemical Co., St. Louis) solution was prepared in saline and passed through a 0.2 μm filter. *Pars plana* intravitreal injections were given through a 30 gauge needle. Injection volume was 0.01 ml in guinea pig and rat, and 0.1 ml in monkey. The same volume of vehicle alone was injected in control eyes. None of these injections caused vitreous hemorrhage or lens damage.

Results

Scotopic ERG

The scotopic ERG waveform from 12 guinea pigs and the intensity–response function are similar to other mammals (Figure 1). b-Wave threshold was near -2.7 log cd/m^2 for 10 μV criterion, and the a-wave first appeared 3.2 log units higher (0.5 log cd/m^2, 10 μV criterion). These are slightly higher than rat which has the b-wave threshold near -3.5 log cd/m^2 and a-wave near -0.7 log cd/m^2 (data not shown). The guinea pig is unusual in having a prominent cone-driven x-wave with white stimulus flashes, whereas the primate x-wave normally requires red stimuli [21].

Some investigators have reported that the guinea pig has an unusual electronegative response when fully dark-adapted. We did not observe such a response in this study (as shown in Figure 1). However, we noticed an electronegative waveform when the guinea pig was not fully dark-adapted (Figure 2). Immediately after the background light was turned off, the guinea pig b-wave was very small, and the overall waveform was electronegative, but only for the first few minutes of dark adaptation. Within 5 min the b-wave was again larger than the a-wave (Figure 2A). Amplitudes of the a- and b-waves returned to fully dark-adapted levels within about 30 min after brief light exposure. All 12 guinea pigs used in this study showed similar response properties. The negative waveform was not observed

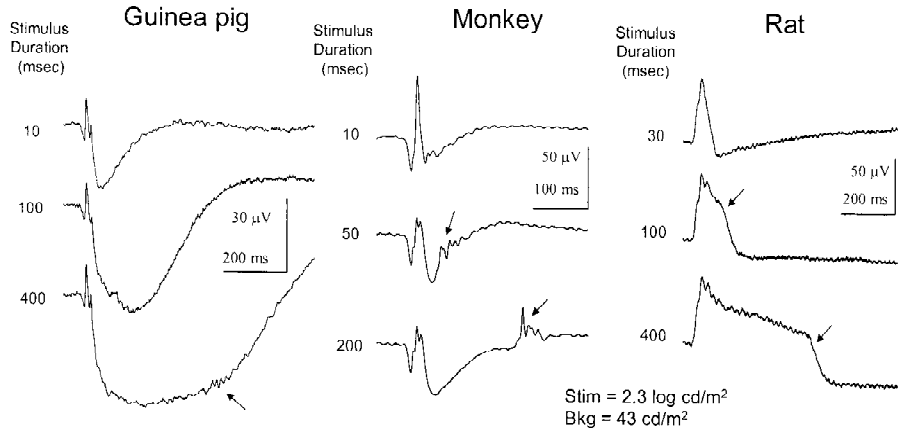

Figure 3. Photopic ERG for different durations of stimulus. The guinea pig has a small a-wave, which is not seen in the rat. Arrows show OFF-wavelets in guinea pig, positive OFF in monkey and negative OFF-response in rat (arrows).

in the two monkeys and the six rats (Figure 2B,C), although the b-wave amplitude decreased dramatically after brief light exposure. The b-wave was always bigger that the a-wave in monkey or rat. We did not observe a scotopic threshold response (STR) with dim stimuli in any of the guinea pigs.

Photopic ERG

Photopic ERG responses are shown in Figure 3. With long duration photopic stimuli, the six E-type rat retinas gave a positive sustained 'square wave' response with essentially no a-wave at stimulus onset. At stimulus termination, the response simply returned rapidly to baseline with no phasic positive d-wave [22]. In contrast, the I-type ERG recorded from the two monkeys shows a large a-wave and has a positive-going phasic d-wave OFF-response at stimulus termination [23]. The photopic ERG of the 12 guinea pigs resembled the primate to some extent and lay intermediate between the I- and the E-type retina. The guinea pig has a small photopic a-wave that is not as prominent as in primate. As in monkey and unlike rat, the waveform was negative and below baseline between the b-wave and the small OFF-wavelets. The guinea pig, like the monkey, showed a positive-going return to baseline at the stimulus termination. The guinea pig lacked a strong phasic d-wave and showed only small OFF-wavelets for longer stimuli.

PDA is a pharmacological tool that blocks the activity of hyperpolarizing bipolar cells (HBC) and horizontal cells (HzC) [24]. In monkey, PDA is known to eliminate the a-wave [23]. In guinea pig, PDA also eliminated the photopic a-wave and enhanced the b-wave (Figure 4). Furthermore, PDA elevated the plateau slightly, although overall it still stayed below the baseline. However, the normal positive-going OFF-wavelets at stimulus termination were replaced with a prominent negative-going transition after PDA. A similar result was observed in the 12 guinea pigs and in the two monkeys. The OFF-responses in both monkey and guinea pig after PDA resemble the normal photopic OFF-response seen in rat without application of drugs.

The suppression of the photopic a-wave in both guinea pig and monkey by PDA supports the idea that second-order HBC/HzC neurons normally contribute to the photopic a-wave in both species [23]. PDA increased the b-wave amplitude in all three species. The right panel in Figure 4 shows PDA sensitive components that were isolated by subtracting the post-PDA waveform from the response before drug injection. The components from all three species are similar in shape, with a sustained negative 'square wave'. Under normal conditions without PDA, this negative component from HBC/HzC activity subtracts from and partially masks the PII b-wave in all three species.

Discussion

Photopic ERG

Granit [25] characterized retinal ERG responses as nocturnal E-type (excitatory) or diurnal I-type (inhibitory). Under photopic conditions, the rat E-type response has a smaller a-wave and a slower b-wave than the I-type monkey response. In response to a

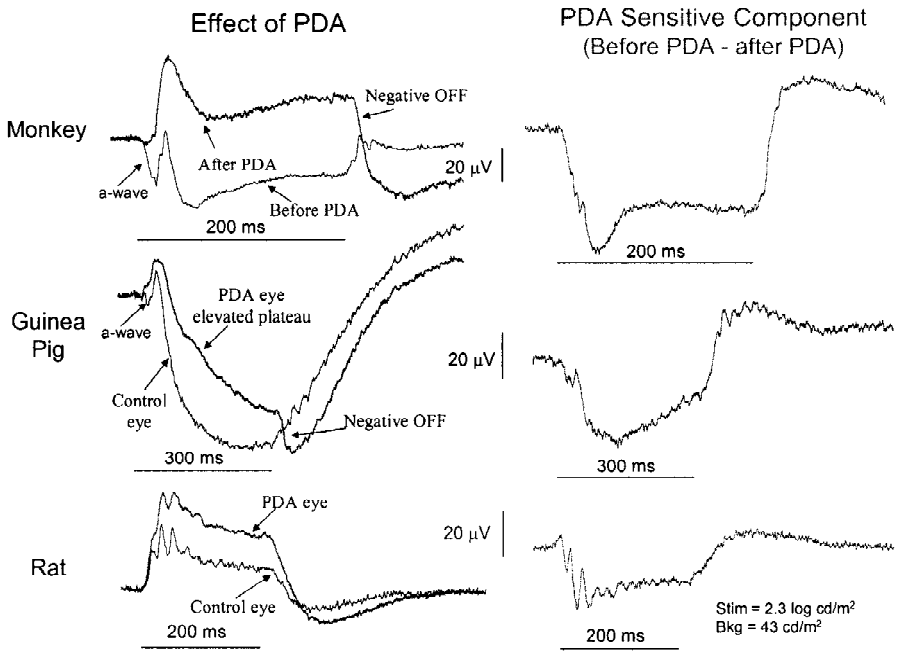

Figure 4. PDA effect on monkey, guinea pig, and rat photopic ERGs. The left column shows the OFF-response in monkey shifted its polarity from positive to negative. The guinea pig developed a negative OFF-responses which was absent before PDA. The rat OFF-response kept its negativity with an increased amplitude. The negative OFF-responses in monkey and guinea pig after PDA resembled that in E-type rat, with and without PDA. The right column shows the PDA sensitive component contains a negative square wave in all three species.

decrement of light, the E-type retina exhibits a corneal negative OFF-response, while the I-type has a positive OFF-response [18, 22, 23, 26]. The behavior of the guinea pig is more like a diurnal I-type monkey than the nocturnal E-type rat.

The features of the guinea pig photopic ERG include: (1) the a-wave is small, and it is depressed by PDA, indicating the contribution of second-order neurons HBC/HzC as in monkey [23]. E-type rat has essentially no photopic a-wave at this intensity level. (2) After PDA, the remaining response exhibits a strong negative-going OFF-response, similar to I-type monkey. While E-type rat normally presents a negative OFF-response, PDA increases the amplitude. These results suggest that the guinea pig has a weak I-type retina, with a photopic ERG intermediate between that of I-type monkey and E-type rat. We term this a 'weak' I-type because the guinea pig lacks a substantial phasic OFF-component.

Early literature suggested that the difference between E-type and I-type retina might depend on the numbers of cones [25]. The guinea pig has more cones than either rat or monkey (Table 1) but shows a response pattern intermediate between both. Evers and Gouras [26] studied the monkey ERG and found that an I-type response seems to require HBC activity. Our PDA result, however, indicates that even the rat has some HBC/HzC contribution in the photopic ERG (Figure 4, right panels), and consequently this alone does not fully explain the rat E-type response.

S-cones in primates and UV-cones in some rodents appear to operate primarily through an ON-center system, whereas the M- and L-cones use both the ON- and OFF-systems. S-cones (including UV-cone) comprise about 5–15% of all cones in many species [10]. Rat has 5–10% UV-cones [8, 27] which is similar to the 5–10% S-cones in primate [28, 29]. Consequently, the proportion of UV or S-cones does not explain the lack of phasic positive OFF-response in the rat. One can further discount the possibility that the strong positive OFF-response in primate results from the existence of long wavelength sensitive L-cones, since the cone-dominant ground squirrel has only a 520 nm M-cone and lacks L-cones [30] but nevertheless shows a prominent d-wave [21].

One explanation for I-type versus E-type waveform is the response timing of HBCs relative to the DBCs. Any timing difference could shape the positive OFF-component, as is certainly the case for the photopic b-wave ON-response in monkey [23], in which

timing differences between DBCs and HBCs contribute counteracting positive-going and negative-going transitions [23]. Figure 4 suggests that a difference in timing for the termination of DBC and HBC activity may also occur at the termination of long stimuli and thereby give the phasic d-wave of primate. Guinea pig and monkey both have a positive-going transition from HBC/HzC activity that can be seen in the PDA isolated component at termination (Figure 4), whereas this transition is smaller and slower in the rat. Consequently, robust HBC/HzC activity at a different time course than DBCs may be important for developing an I-type OFF-response.

Scotopic ERG

Two studies [31, 32] reported that the guinea pig has an electronegative dark-adapted response. We have looked carefully for this phenomenon in our recordings but have not observed it in any of the 12 guinea pigs we studied when they were fully dark-adapted. Our result is consistent with an earlier report addressing this electronegative response in different age groups of guinea pigs [33]. We observed electronegative responses under scotopic conditions only during the early stages of dark-adaptation (Figure 2A) and this electronegative waveform disappeared with further dark adaptation.

In considering a cause of the electronegative response in the previous reports, we wondered whether light-rearing conditions could affect the white guinea pig which has a pale iris color similar to the albino rat that is susceptible to damage from light exposure. We tested this possibility by exposing four guinea pigs to 2000 lux fluorescent light for 24–48 h, using the same light-damage procedure that we used in albino rats [34]. Two days after light exposure, the ERG did not show a negative waveform in these animals. We have not explored whether the type of anesthetic might make any difference, and cannot explain the discrepancy between these studies of the guinea pig ERG.

Although our laboratory has successfully recorded an STR in many species including rat, cat, monkey, and human [34, 35], we could not identify an STR in the guinea pig. In the rabbit the threshold response is positive-going [36] which could be due to the inverted vascular organization of the rabbit retina, in which the major blood vessels lie underneath the retina rather than on the surface as in primate, rodent and cat. The Müller cell potassium conductance is different in rabbit compared to cat and monkey [37], and this may contribute to the presence or absence of an STR. The guinea pig has an avascular retina like the rabbit [38], although the polarity of Müller cell potassium conductance in the guinea pig is not known.

Table 2. Comparison of ERG in rat, mouse, guinea pig, monkey and human

	STR	Photopic a-wave	Photopic d-wave
Mouse, rat	Yes	Small or no	E-type
Guinea pig	No	Yes	Weak I-type
Macaque monkey	Yes	Yes	I-type
Human	Yes	Yes	I-type

We briefly compared the ERG in rat, guinea pig, monkey and human (Table 2). In summary, the guinea pig has a weak I-type ERG that has some similarity to the primate I-type responses and it appears to be a suitable animal for testing glutamate analog pharmacology effects on the ERG. However, the guinea pig can not fully substitute for the monkey in primate photopic ERG research.

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