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Multifocal Electroretinogram in Rhodopsin P347L Transgenic Pigs

Yiu-fai Ng¹, Henry H. L. Chan¹, Patrick H. W. Chu¹, Chi-ho To¹, Brian C. Gilger², Robert M. Petters³, and Fulton Wong^{4,5}

¹ Laboratory of Experimental Optometry (Neuroscience), School of Optometry, The Hong Kong Polytechnic University, Hong Kong SAR, China

² Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

³ Department of Animal Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, North Carolina

⁴ Department of Ophthalmology, Duke University, Durham, North Carolina

⁵ Department of Neurobiology, School of Medicine, Duke University, Durham, North Carolina

Abstract

Purpose—Neural ectopic rewiring in retinal degeneration such as retinitis pigmentosa (RP) may form functional synapses between cones and rod bipolar cells that cause atypical signal processing. In this study, the multifocal electroretinograms (mfERGs) of a large animal model of RP, the rhodopsin P347L transgenic (Tg) pig, were measured to examine the sources and nature of altered signal processing.

Methods—mfERG responses from a 6-week-old Tg pig were recorded before and after sequential application of tetrodotoxin (TTX), *N*-methyl-D-aspartate (NMDA), 2-amino-4-phosphonobutyric acid (APB), and *cis*-2,3-piperidinedicarboxylic acid (PDA), to identify contributions to the retinal signal from inner retinal neurons, the ON-pathway, the OFF-pathway, and photoreceptors. The mfERG response contributions from different retinal components of in the Tg eyes were estimated and compared with control data from eyes of age-matched wild-type (WT) pigs.

Results—There was a prominent difference in the estimates of the inner retinal response and ON-bipolar cell pathway contribution between the Tg and WT mfERG responses. In particular, the early components of the inner retinal contribution were obviously altered in the Tg mfERG. The inner retinal components at approximately 24 and 40 ms appeared to be inverted. Differences in the estimates of OFF-bipolar cell pathway contributions were minimal. There was no change in cone cell responses in the Tg mfERG.

Conclusions—In Tg retinas, ectopic synapses formed between cones and rod bipolar cells probably altered signal processing of the ON-bipolar cell pathway. In response to the altered visual signal input from the outer retina, signal processing in inner retinal neurons was also modified.

Photoreceptor cell death is common in many retinal diseases, such as age-related macular degeneration (ARMD), Stargardt's disease, Leber's congenital amaurosis, and retinitis pigmentosa (RP). Different animal models have been developed to study these retinal diseases,^{1–4} and histologic evidence of neural rewiring and ectopic synaptogenesis (involving surviving

Corresponding author: Henry H. L. Chan, School of Optometry, The Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR, China; henryhl.chan@polyu.edu.hk.

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photoreceptors and bipolar cells) concomitant with or after photoreceptor cell death has been reported. Ectopic synaptogenesis occurs in the degeneration of rods^{5–8} and cones^{9,10} and in the absence of rods during development.^{8,11} In the present study, we investigated the retinal response alterations in a model known to have ectopic synapses between surviving cones and rod bipolar cells.

When the visual signal is transmitted from the outer plexiform layer to the inner retina via ectopic synapses, an inner retina response to the altered visual input may occur. Thus, an electrophysiological method is needed to examine changes in response of photoreceptors, bipolar cell pathways, and inner retinal neurons.

The multifocal electroretinogram (mfERG) is an advanced visual electrodiagnostic technique that can measure responses from multiple retinal regions.¹² mfERG recordings obtained from primates before and after the injection of pharmacologic agents that block the activities of specific neural circuits have been used to identify the contributions of different retinal pathways to the response.^{13,14} We adopted this strategy to define the retinal signal contributions of different retinal pathways of wild-type pigs.¹⁵

The porcine eye is anatomically similar in size to the human eye,^{16,17} and, compared with the rodent eye, its retina is relatively cone photoreceptor rich.^{16,18,19} Its cone-to-rod ratio at the optic streak is very close to that of the central human retina. The color of the optic streak and fundus is orange to pale gray with pigmented epithelial cells. This is very similar to the human eye, and there is no retinal tapetum in the porcine eye.²⁰ Thus, porcine eye models are suitable for ERG assessments (e.g., full-field ERG and mfERG). The mfERG allows the selection or grouping of signals from the optic streak,¹⁶ which is expected to have a cone density similar to that of the paramacular region of the human retina.²¹ In addition, the mfERG can measure inner retinal activity, which has been shown in primates,^{13,14} and it is believed that the mfERG can help to investigate localized retinal defects as well as changes from outer and inner retinal layers.

In the present study, we defined mfERG changes in the P347L transgenic (Tg) pig, which is a large animal model known to have ectopic synaptogenesis between cones and rod bipolar cells⁶ and altered retinal electrical response.²² The ectopic synaptic connection between rod bipolar cells and cone pedicles is thought to alter the physiology of the outer retina and to affect signal processing in the inner retina. Although the mfERG theoretically provides information only on cone pathways, it is believed that the signal processing in the cone pathway will be altered because of ectopic synaptogenesis involving both rod and cone pathways.^{5,6}

Materials and Methods

In this study, the mfERG of the Tg and wild-type (WT) pigs¹⁵ were compared. The retinal cellular contribution of the WT porcine mfERG has recently been published,¹⁵ and the data of the WT mfERG have been used in this study, to compare with the findings of the Tg mfERG. The applications of pharmacologic agents known to block activities of specific neural circuits have identified and defined the contributions of specific retinal pathways in shaping the mfERG response.^{13–15} The pharmacologic agents used include isoflurane (ISO), *N*-methyl-D-aspartate (NMDA), tetrodotoxin (TTX), 2-amino-4-phosphonobutyric acid (APB), and *cis*-2,3-piperidinedicarboxylic acid (PDA). ISO is an anesthetic agent, and TTX is a voltage-gated sodium channel blocker. They inhibit the voltage-gated sodium channel-triggered action potential in third-order neurons, such as ganglion cells and amacrine cells. NMDA is an ionotropic glutamatergic receptor agonist that depolarizes cells with NMDA receptors, such as ganglion cells and amacrine cells. APB is a glutamate analogue that blocks signal transmission from photoreceptors to ON-bipolar cells. PDA is a glutamate analogue that blocks

transmission from photoreceptors to OFF-bipolar cells and horizontal cells and transmission from ON- and OFF-bipolar cells to ganglion cells and amacrine cells. Previously, we reported the activities of different retinal cell types of WT¹⁵ by application of different combinations of those pharmacologic agents. The combined application of ISO+TTX+NMDA essentially eliminates the activities of inner retinal neurons. Application of APB after ISO+TTX+NMDA also inhibits ON-bipolar pathway activity, and the application of PDA after ISO+TTX+NMDA inhibits OFF-bipolar pathway activity in the retina. The application of ISO+TTX+NMDA +APB+PDA inhibits most of the activity of retinal cell types except the photoreceptor response to light stimulation. By subtraction of responses measured under these various conditions, the responses of photoreceptors, outer retina, inner retina and ON- and OFF-bipolar pathways can be estimated. The differences of retinal activities of Tg and WT were investigated in this study.

Animals

mfERG were obtained from 16 eyes of eight 6-week-old Tg Yorkshire pigs. The normal pig data used for comparison were obtained from 14 eyes of ten 6-week-old WT Yorkshire pigs. Before anesthesia, the animals were fasted for 12 hours; initial anesthesia consisted of ketamine (20 mg/kg IM) with xylazine (2 mg/kg IV) and propofol (14–20 mg/kg/hr IV). The pupils were dilated with topical tropicamide (1%) and phenylephrine (10%); the cornea was anesthetized with topical proparacaine HCl (1%). After the collection of mfERG control data, propofol anesthesia was replaced with isoflurane (ISO; 4%) with 100% oxygen for the other parts of the experiment. ISO anesthetic is the standard one used in veterinary surgical procedures, and it is suitable for prolonged anesthetic in large animals. After orotracheal intubation, artificial ventilation was used to maintain the blood SpO₂ level at 95% to 100%. The heart rate was monitored throughout the experiment. Throughout anesthesia, lactated Ringer's solution was administered IV, and rectal temperature was maintained at 38°C to 39°C with a circulating hot water heating pad and blanket. All experimental and animal handling procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, as well as the institutional guidelines approved by North Carolina State University Institutional Animal Care and Use Committees, and the Animal Ethics Subcommittee of The Hong Kong Polytechnic University.

Multifocal Electroretinogram

Two subdermal electrodes (Grass F-E7; Astro-Med, Inc., West Warwick, RI) were placed subcutaneously at the temporal canthus and at the rostrum, as reference and ground electrodes, respectively. An ERG jet contact lens monopolar electrode (Universal SA, La Chaux-de-Fons, Switzerland) was placed on the cornea with lubricant gel (0.3% Lacryvisc; Alcon, Cleveland, OH) as the active electrode. A stimulus pattern, with 103 nonscaled white (160 cd/m²) and black (1 cd/m²) hexagons (Fig. 1B) and a gray peripheral background (80 cd/m²), was displayed on a 17-in. CRT monitor (model s7540; Hewlett-Packard, Palo Alto, CA) with a refresh rate of 85 Hz (11.7 ms per frame) for mfERG measurement. The mfERG stimulation was driven by a visual evoked response imaging system (VERIS 5.01 software; Electro-Diagnostic-Imaging; San Mateo, CA) according to a pseudorandom binary m-sequence of $2^{14} - 1$, providing 3.2 minutes of recording, which is sufficient to minimize random noise.¹² The signals generated were amplified (Grass model CP122; Astro-Med, Inc.) with a gain of 20,000 and bandpass from 1 to 300 Hz before processing by the VERIS system. This study focused on the first-order kernel responses, which have been reported to include both inner and outer retinal activity.^{13,14}

To control ocular alignment and prevent drift of ocular orientation during mfERG recording, we placed two conjunctival sutures approximately 2 mm from the superior and inferior limbal margins. Alignment of the eyeball to the stimulus pattern was facilitated by using the trace arrays of localized mfERG responses. Retinal features, such as optic nerve head and optic

streak, were identified as the basis for alignment (Fig. 1A). The alignment of the eyeball to the stimulus pattern was checked after each intravitreal drug injection. Refractive error was corrected by using ophthalmic lenses placed in front of the eye under test after retinoscopy had been performed with the contact lens electrode in place.

Intravitreal Injections

After acquisition of mfERG data under propofol and ISO, pharmacologic agents (all from Sigma-Aldrich, St. Louis, MO) including NMDA, TTX, APB, and PDA were administered to rats under ISO anesthesia; 25 μ L of each agent was injected (using 28-gauge needles) separately into the vitreous, 3 mm above the superior limbus, to achieve 4 mM, 5 μ M, 1 mM, and 3.5 mM vitreous concentrations based on an estimated vitreous volume of 2.0 mL for each eye. The concentrations of pharmacologic agents were sufficient to achieve the desired effects in other animals^{14,23} and in porcine mfERGs^{15,24,25} (Tremblay F, et al. *IOVS* 2005;46:ARVO E-Abstract 2247). Sixteen eyes of eight Tg pigs were used. TTX+NMDA were injected into all eyes after mfERGs were recorded with the animals under ISO. APB and PDA were then injected into both eyes before mfERG measurements; PDA and APB were applied again to the opposite eyes (so that, ultimately, each eye had both APB and PDA applied) before additional mfERG measurements. In the control group, 14 eyes of 10 WT pigs were used. Both eyes of four WT pigs underwent the same procedures as those of the Tg pigs. In the other six WT pigs, only one eye was used (three right eyes for APB and three left eyes for PDA before the APB+PDA applications).

After conjunctival suturing and each intravitreal injection, binocular indirect ophthalmoscopy was performed to ensure retinal integrity. The mfERG was recorded approximately 90 minutes after administration of each drug.

Analysis

The mfERG responses from 103 individual responses were grouped by region: regions with p1 (peak-to-peak) amplitude in the top 25th percentile were grouped as the response from the optic streak, whereas the other regions (p1 amplitude not within the top 25th percentile) were grouped as the area outside the optic streak. The waveform features (Fig. 1C) were defined conventionally, according to Lalonde et al.²⁴; details are given by Lalonde et al. and Ng et al.¹⁵ Amplitude and implicit time of the mfERG responses of Tg pigs before and after applications of ISO, TTX, NMDA, APB, and PDA were measured and compared with the control data of WT pigs.¹⁵ In addition, the inner retinal mfERG responses of WT and Tg pigs were compared. Repeated-measures, one-way ANOVA with the Tukey post hoc test was used for statistical analysis.

Results

The first-order kernel mfERG waveforms at the optic streak from Tg and WT retinas in pigs under propofol and ISO anesthesia are shown in Figure 2, and the amplitudes and implicit times are listed in Tables 1 and 2. The Tg mfERG amplitude was generally smaller than that of the WT. In the baseline data (under propofol anesthesia), the amplitude of the Tg mfERGs were significantly smaller than the WT mfERG for the p1 ($P < 0.01$), n2 ($P < 0.001$), and p2 ($P < 0.001$) components. The implicit time of p2 was also significantly shorter ($P < 0.05$) in the Tg pig. Under ISO anesthesia, although there were no significant differences in amplitude or implicit time between the Tg and WT mfERG, the p1 and n2 amplitudes were somewhat smaller in the Tg mfERG.

Figure 3A shows the waveforms of the first-order kernel after the application of different combinations of TTX, NMDA, PDA, and APB under ISO anesthesia. The amplitudes and

implicit times are listed in Tables 1 and 2. After TTX+NMDA application, the difference between the Tg and WT mfERG waveforms was reduced. For the Tg mfERG, n1 and p1 amplitudes showed small reductions under TTX+NMDA, and p2 was significantly increased ($P < 0.05$) compared with the WT mfERG. After PDA injection, there were no significant changes in amplitude or implicit time of Tg and WT mfERG, but the n1 and p1 amplitudes were relatively smaller in the Tg mfERG, and the p2 amplitude was relatively larger in the Tg mfERG. After APB injection, there were no significant amplitude changes in the Tg mfERG, but the implicit time of n1 was shortened significantly ($P < 0.05$). After APB+PDA had been injected together, apart from a significant delay of the n2 implicit time ($P < 0.001$) in the Tg mfERG, there were no significant differences in amplitudes or implicit times between the two groups.

Figure 3B shows the estimates of difference among the waveforms between the Tg and WT mfERGs. The estimated waveforms were obtained by subtracting the waveform before drug application from that after drug application. The subtraction of waveforms was performed using the visual evoked imaging system (VERIS; EDI), a method that has been used in previous studies.^{13,14,26} These waveforms show components of the mfERG that were altered in the Tg mfERG. There was a loss of a corneal negative–positive–negative component after TTX+NMDA and a positive–negative component after PDA application. However, there was only a loss of small oscillations after APB application, and there was almost no difference between the two types of mfERG after all drugs had been injected.

The estimates of response of the inner retinal elements in the Tg and WT mfERGs shown in Figure 4 were markedly different. The estimates were produced by subtraction of the waveforms under ISO anesthesia after TTX+NMDA application from those under propofol anesthesia. The major features of the response were oscillatory wavelets, arbitrarily named OW1, OW2, and OW3. OW1 and OW2 in Tg mfERGs showed opposite characteristics from those in WT. The amplitudes and implicit times are summarized in Tables 3 and 4. There were significant differences of amplitudes of OW1 ($P < 0.001$) and OW2 ($P < 0.001$) between the Tg and WT mfERGs, but no difference in OW3.

Discussion

Recently, there has been increasing histologic evidence of neural rewiring, such as ectopic synaptogenesis, after photoreceptor cell death.^{5–10} The P347L Tg pig is an important large animal model shown to have ectopic synaptogenesis between cones and rod bipolar cells⁶ and altered retinal electrophysiological response.²² Six-week-old P347L Tg pigs were chosen for our study because of their relatively spared cone physiology with extensive loss of rods.¹ A previous study using flash ERG found that the Tg and WT cone response amplitudes were the same, whereas there was no detectable rod-mediated b-wave in 4-week-old Tg animals.²² Our study further demonstrated signal alterations in the Tg pigs in both outer and inner retinal responses, most likely as a consequence of retinal neural rewiring.

Although there has been no direct evidence of different glutamate receptor types in porcine retina, Peng et al.⁶ reported the presence of GoG protein, which is related to mammalian metabotropic glutamate receptor 6 in Tg rod bipolar cells after ectopic synaptogenesis. Furthermore, the effects of PDA and APB on WT porcine mfERGs¹⁵ suggested the presence of corresponding receptors in the porcine retina. In addition, the PDA- and APB-sensitive waveforms or components in porcine mfERGs¹⁵ resemble those of primates.^{13,14} All these findings suggest that the porcine retina is a viable alternative to primate retina for dissecting the cellular contributions to the retinal signal using the mfERG.

It is unlikely that the observed mfERG alterations are caused by transient increase of intraocular pressure or mechanical effects of injection, because the injection volume of the pharmacologic solution in normal saline form was referenced to that of injection in monkey^{13,14} and porcine eyes.²⁴ Furthermore, we tested earlier the effect of normal saline injection on perfused isolated porcine eyes and found no alteration of the mfERG with the same volume of normal saline injection.

Outer Retinal Signal Alteration in the Tg mfERG

In animals under propofol anesthesia, the Tg mfERG was smaller than the WT (Fig. 2A). This result agrees with most of the studies of patients with RP.^{27–31} The mfERG contributed by the outer retina can be estimated after the removal of inner retinal activity by administration of ISO+TTX+NMDA (Fig. 3A, top trace). We have shown that ISO removes only part of the inner retinal contribution in the porcine mfERG,^{15,24} and the PDA and APB sensitive elements under ISO were well preserved.¹⁵ Accordingly, signals originating from cells affected by photoreceptor degeneration and synaptic rearrangement, such as surviving cone photoreceptors in contact with rod bipolar cells, should be detected. The estimate of outer retinal mfERG response is mainly a summated contribution from ON- and OFF-bipolar cell pathways.

The changes in the outer retinal response as a consequence of rod photoreceptor cell death between the Tg and WT porcine mfERGs are illustrated by subtracting the outer retinal elements of the Tg mfERG from those of the WT mfERG (Fig. 3B). Although the outer retinal Tg mfERG approximated the WT (Fig. 3A, top trace), a corneal negative–positive–negative component appeared to be lost in the Tg (Fig. 3B, top trace), demonstrating that outer retinal activity in the Tg eye is not identical with that in the WT. It may be caused by the difference in implicit times between the outer retinal responses of the Tg and WT eyes. When OFF-bipolar cells were blocked, the Tg mfERG seemed to have a loss of a corneal positive component followed by a corneal negative wavelet (Fig. 3B, second trace). When ON-bipolar cells were blocked, a series of small oscillating wavelets following a corneal negative component were likely to be diminished in the Tg mfERG (Fig. 3B, third trace).

In Tg pigs, a substantial number of cones remain¹ and there is no significant loss of rod bipolar cells after extensive rod degeneration.⁶ Consistent with these morphologic data, mfERG findings showed no conspicuous waveform change in the Tg cone response (Fig. 3B, 4th trace). The mfERG findings are also consistent with the absence of significant difference in the isolated Tg and WT cone responses, as reported by Banin et al.²²

Therefore, the source of waveform difference between Tg and WT mfERGs at the level of the outer retina seemed to arise mainly from the ON-bipolar cell pathway (Fig. 3B, second trace), with a small contribution from the OFF-bipolar cell pathway (Fig. 3B, third trace).

Neural rewiring and ectopic synaptogenesis are important features in the degenerating retina and the synaptogenesis starts early in neural retina development^{7,8,11} or after the onset of rod cell degeneration.^{5,6,10} After they form the ectopic contacts with cone photoreceptors, the rod bipolar cells retain the characteristics of ON-bipolar cells^{6,32} and thus probably continue to use the mGluR6-mediated signaling pathway, which may explain why the waveform difference between the Tg and WT mfERG at the level of the outer retina occurs mainly in the ON pathway.

Marc et al.³³ recently reported increased ionotropic glutamate receptor response (OFF-response) and loss of rod bipolar cell signatures in a human RP retina. However, in our data, the Tg showed an alteration largely in the ON-bipolar cell pathway response, with minimal alteration in OFF-bipolar cell pathway response. The difference between our findings and those of Marc et al.³³ may be due to differences in the stages of retinal degeneration studied.

Inner Retinal Signal Alteration in Tg mfERG

In rod-ablated retina, cellular components of the rod-specific signal pathway downstream from rods may persist long after rod degeneration. The altered signaling in the ON pathway as observed at the level of the outer retina could in turn cause alterations in the inner retina.

In this study, ISO would partially affect the inner retinal activity and the further application of TTX+NMDA would suppress nearly all inner retinal activity.^{13,34} However, some types of inner retinal neurons that do not process NMDA receptors³⁵ rather involve GABA receptors. We did not use GABA for the suppression of inner retinal activity, because the use of GABA could affect the outer retina.³⁵ According to Hood et al.,¹³ despite the difference in action of NMDA and GABA, the effects of TTX+NMDA are sufficient to suppress inner retinal activity.

The estimates of inner retinal elements of Tg and WT porcine eyes were obtained by subtracting their mfERG responses after administration of ISO+TTX+NMDA from those before administration of ISO+TTX+NMDA (Fig. 4). Both Tg and WT mfERGs showed waveforms with oscillatory wavelets OW1, OW2, and OW3 at approximately 24, 40, and 64 ms, respectively. No signal filtering was used to extract the OWs from the mfERGs. Thus, they may not be the same as the oscillatory potentials of the full-field ERGs.³⁶ These oscillatory wavelets or components are attributable to inner retinal cells because they were removed by ISO+TTX+NMDA, which presumably inhibited or removed most of the inner retinal contribution. Of note, the inner retinal responses were corneal positive–negative–positive (Fig. 4, top trace) in the WT but were corneal negative–positive–positive (Fig. 4, middle trace) in the Tg mfERG. OW3 was very similar in both the Tg and WT mfERGs. Its implicit time matched that of p3 of the normal porcine mfERG, which is reported to be eliminated by optic nerve sectioning.²⁴ As the Tg pig showed altered OW1 and OW2, but no change in OW3, the involvement of the optic nerve due to retinal dystrophy in the Tg eye is thought to be minimal. This notion agrees with the preservation of retinal ganglion cells in young patients with RP.³⁷ It may be that insufficient time has elapsed after the death of the photoreceptors for marked transneuronal degeneration to occur at the inner retinal level in this animal model.³⁸

Therefore, at the level of the inner retina, the difference between the estimates of response of WT and Tg retinas are reflected in the changes in OW1 and OW2 (Fig. 4). Although the cellular origins of these wavelets are not known, these changes in the inner retina are probably due to alterations in synaptic circuitry induced by altered activity from the outer retina.

Although there was small number of significant differences between Tg and WT porcine mfERGs in this study, the results pointed to specific retinal pathways and regions that were altered in Tg pigs. These results were made possible by combining the mfERG technique with sequential application of pharmacologic agents with known effects on the retina. The difference in the ON pathway in Tg compared with WT retinas is likely to arise because of ectopic synaptogenesis involving cone photoreceptors and rod bipolar cells, and the differences in OW1 and OW2 are probably the result of adaptive responses by the inner retinal neurons to altered input from the outer retina. The neural rewiring in degenerating retina may be an example of neural plasticity that provides dynamic and adaptive changes to maintain the integrity of the retina despite profound loss of photoreceptors. In addition, the inner retinal neurons and their connections may be modified by the light-stimulated signal from the outer retina to make the remaining neural circuits more suitable for the cone-mediated pathways.

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Figure 1.

(A) mfERG first-order trace array from the right eyes of Tg pig 2 and WT* pig 4 under propofol anesthesia. The location with lowest response was the landmark of optic nerve head and reference point assisting alignment of stimulator. (B) One hundred three nonscaled hexagonal stimulus pattern of mfERG. (C) First-order kernel (K1) waveform of averaged mfERG from WT* pig 4 right eye under propofol anesthesia. *WT mfERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Figure 2.

First-order kernel (K1) waveform of averaged mfERG from both eyes of WT* pig 4 (*gray*) and Tg pig 2 (*black*) under propofol (**A**) and ISO (**B**) anesthesia. The p1, n2, and p2 of the Tg K1 waveform were smaller than that of the WT. (*A, dotted trace*) The difference between the averaged mfERG of all WT* and Tg porcine eyes. *WT mfERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Figure 3.

(A) First-order kernel (K1) waveform of mfERG from WT* pig 4 (gray) and Tg pig 2 (black) after TTX, NMDA, PDA, and APB administration under ISO anesthesia. The traces under the effect of ISO+TTX+NMDA and APB+PDA were the averages of both eyes from WT* pig 4 and Tg pig 2. The traces under the effects of APB and under those of PDA were from the right eye and the left eye of WT* pig 4 and Tg pig 2, respectively. There was a positive peak at 57 ms in the K1 waveform of Tg mfERG but not in the WT mfERG after PDA administration. (B) The estimates of difference between the K1 waveforms of averaged mfERG of all Tg and WT* porcine eyes. *WT mfERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Propofol - (ISO+TTX+NMDA)

Figure 4.

The averaged estimates of inner retinal response from both eyes of the WT* pig 4 and Tg pig 2. There were three oscillating wave features arbitrarily named OW1, OW2, and OW3. The OW1 and OW2 were altered in the Tg mERG. *Bottom trace*: the difference between the estimates of the inner retinal element of all WT* and Tg porcine eyes. *WT mERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Table 1
Mean Amplitude (nV/degree2) of First-Order Kernel Traces with SEM of mERG at the Optic Streak Area

Amplitude	First-Order Kernel (K1)			
	n1	p1	n2	p2
Propofol				
WT [†]	4.89 (0.63)	22.24 (2.75)	32.92 (3.99)	16.89 (1.92)
Tg	3.71 (0.38)	11.21 (1.15)	14.66 (1.60)	8.06 (1.06)
ISO				
WT [†]	6.27 (0.79)	19.30 (2.10)	21.99 (2.14)	6.34 (0.90)
Tg	5.80 (0.55)	13.53 (1.28)	12.17 (1.34)	5.51 (0.64)
ISO+TTX+NMDA				
WT [†]	4.33 (1.24)	21.15 (3.08)	28.30 (4.37)	12.74 (1.88)
Tg	3.17 (0.82)	19.56 (2.62)	28.85 (3.49)	16.51 (2.33)
ISO+TTX+NMDA+APB				
WT [†]	7.11 (0.88)	11.24 (1.26)	6.76 (0.96)	2.83 (0.67)
Tg	7.45 (1.23)	11.82 (1.80)	6.82 (1.10)	1.93 (0.42)
ISO+TTX+NMDA+PDA				
WT [†]	0.98 (0.30)	21.13 (3.78)	33.23 (5.67)	13.33 (1.64)
Tg	0.66 (0.26)	18.90 (5.11)	32.77 (9.22)	19.10 (5.89)
ISO+TTX+NMDA+APB+PDA				
WT [†]	4.56 (0.76)	5.81 (0.93)	1.82 (0.40)	0.40 (0.08)
Tg	5.07 (0.97)	7.00 (1.37)	2.89 (0.62)	1.55 (0.92)

Probabilities indicate significant difference from the wild type mERG.

* $P < 0.05$.

** $P < 0.01$

*** $P < 0.001$ indicate significant difference from the wild type mERG.

[†] WT mERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Table 2
Mean Implicit Time (ms) of First-Order Kernel Traces with SEM of mfERG at the Optic Streak Area

Implicit Time	First-Order Kernel (K1)				
	n1	p1	n2	p2	p3
Propofol					
WT [†]	12.37 (0.22)	27.07 (0.41)	43.18 (0.55)	56.98 (1.07)	69.98 (2.64)
Tg	12.11 (0.24)	23.56 (0.89)	41.40 (0.48)	53.13 (0.79) *	65.14 (1.04)
ISO					
WT [†]	13.01 (0.22)	28.61 (0.39)	43.84 (0.67)	52.71 (0.39)	69.29 (1.52)
Tg	13.06 (0.35)	26.19 (0.96)	42.53 (1.29)	53.12 (1.43)	72.66 (2.74)
ISO+TTX+NMDA					
WT [†]	12.93 (0.50)	26.76 (0.71)	43.08 (0.43)	54.98 (0.50)	74.55 (1.09)
Tg	11.81 (0.36)	25.22 (0.61)	41.85 (0.33)	55.79 (0.61)	77.60 (1.39)
ISO+TTX+NMDA+APB					
WT [†]	18.91 (0.79)	35.29 (0.58)	47.57 (0.94)	58.90 (0.98)	80.33 (2.60)
Tg	16.90 (0.96) *	33.68 (1.75)	46.53 (2.03)	55.97 (1.79)	71.15 (1.82)
ISO+TTX+NMDA+PDA					
WT [†]	8.83 (0.47)	26.22 (0.87)	43.97 (0.82)	59.28 (1.11)	82.36 (3.97)
Tg	8.83 (0.79)	25.09 (0.57)	42.30 (0.35)	56.59 (0.82)	77.02 (2.19)
ISO+TTX+NMDA+APB+PDA					
WT [†]	4.68 (1.31)	42.80 (2.20)	51.74 (1.81) ***		
Tg	26.33 (1.16)	45.36 (1.36)	65.28 (2.76) ***		

Probabilities indicate significant difference from the wild type mfERG.

* $P < 0.05$.

*** $P < 0.001$.

[†] WT mfERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Table 3

Mean Amplitude (nV/degree²) of First-Order Kernel Traces with SEM of Inner Retinal Elements at the Optic Streak Area

Amplitude		Inner Retinal Elements		
		OW1	OW2	OW3
Propofol - (ISO+TTX+NMDA)	WT [†]	8.19 (4.48)	14.39 (6.71)	6.28 (1.06)
	Tg	-7.71 (2.60)***	-10.31 (3.27)***	4.19 (0.38)

Probabilities indicate significant difference from the wild-type mfERG.

*** $P < 0.001$.

[†] WT mfERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.

Table 4

Mean Implicit Time (ms) of First-Order Kernel Traces with SEM of Inner Retinal Elements at the Optic Streak Area

Implicit Time		Inner Retinal Elements		
		OW1	OW2	OW3
Propofol+ISO+TTX+NMDA	WT [*]	23.89 (1.27)	40.25 (1.05)	63.85 (1.15)
	Tg	24.72 (1.12)	41.30 (1.19)	64.65 (0.79)

^{*} WT mfERG data reprinted with kind permission of Springer Science and Business Media from Ng Y, Chan HHL, Chu PHW, et al. Pharmacologically defined components of the normal porcine multifocal ERG. *Doc Ophthalmol*. Published online August 25, 2007.