Article

Time-Dependent Decline in Multifocal Electroretinogram Requires Faster Recording Procedures in Anesthetized Pigs

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Purpose: The time-dependent effect of anesthetics on the retinal function is debated. We hypothesize that in anesthetized animals there is a time-dependent decline that requires optimized multifocal electroretinogram (mfERG) recording procedures.

Methods: Conventional and four-frame global-flash mfERG recordings were obtained approximately 15, 60, and 150 minutes after the induction of propofol anesthesia (20 pigs) and isoflurane anesthesia (nine pigs). In six of the propofol-anesthetized pigs, the mfERG recordings were split in 3-minute segments. Two to 4 weeks after initial recordings, an intraocular injection of tetrodotoxin (TTX) was given and the mfERG was rerecorded as described above. Data were analyzed using mixed models in SAS statistical software.

Results: Propofol significantly decreases the conventional and global-flash amplitudes over time. The only significant effect of isoflurane is a decrease in the global-flash amplitudes. At 15 minutes after TTX injection several of the mfERG amplitudes are significantly decreased. There is a linear correlation between the conventional P1 and the global-flash DR mfERG-amplitude ($R^2 = 0.82$, slope = 0.72, $P < 0.0001$). There is no significant difference between the 3-minute and the prolonged mfERG recordings for conventional amplitudes and the global-flash direct response. The global flash–induced component significantly decreases with prolonged mfERG recordings.

Conclusions: A 3-minute mfERG recording and a single stimulation protocol is sufficient in anesthetized pigs. Recordings should be obtained immediately after the induction of anesthesia. The effect of TTX is significant 15 minutes after injection, but is contaminated by the effect of anesthesia 90 minutes after injection. Therefore, the quality of mfERG recordings can be further improved by determining the necessary time-of-delay from intraocular injection of a drug to full effect.

Translational Relevance: General anesthesia is a possible source of error in mfERG recordings. Therefore, it is important to investigate the translational relevance of the results to mfERG recordings in children in general anesthesia.

Introduction

The use of anesthetic agents is a challenge when assessing retinal function with the multifocal electroretinogram (mfERG) in experimental animal models. Several studies have shown that electroretinogram amplitudes are affected by anesthesia.[1–6] However, since mfERG is one of the best methods to examine retinal function in vivo we find it relevant to explore and minimize the effect of anesthetic agents. Theoretically, it is possible to reduce the effect of anesthesia on mfERG by altering the concentration, agent, or duration.[5,7,8] In reality, the anesthetic concentration in animal experiments is difficult to adjust, as it must be sufficient to induce pain relief, amnesia, sleep, and muscle relaxation. Thus, a change in duration and agent are possible ways to minimize the effect of anesthesia on mfERG.

The duration of anesthesia can be reduced by shortening the duration of a single recording (number of stimulations). Currently, a duration of at least 8

minutes for 103 hexagon mfERG recordings is recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV).[9] In experimental studies recordings typically last from 3 to 14 minutes.[2,3,6,10–18] The balance between the signal-to-noise ratio and the exhaustion of the examined individual determines the necessary duration of a recording. It is likely that when animals are anesthetized the noise is minimal and shorter recordings would be sufficient.

The duration of anesthesia can be reduced further by restricting the number of different mfERG recordings (stimulation protocols). Commonly, the conventional and four-frame global-flash mfERG stimulation protocols are used. A conventional mfERG is produced by a pseudorandom stimulation of individual retinal areas by light (flash) and dark hexagons.[19] The global-flash mfERG is obtained by a four-frame stimulation consisting of a conventional multifocal frame, a full-field dark frame, a full-field flash frame, and another dark frame.[12] The response to an immediately preceding flash represents outer retinal function and is displayed as the conventional P1 amplitude (first order kernel) and the global-flash direct response (DR).[12,20,21] Retinal adaptation to successive mfERG flashes represents inner retinal function and is displayed in the conventional second order kernel and the global flash–induced component (IC).[12,20,22–30] Therefore, it is reasonable to believe that the amplitudes of the two stimulation protocols have the same origin and would be affected in a similar way by the same alterations. In this study, we will block inner retinal function with tetrodotoxin (TTX).[3,4,10,17,31–34] With the use of TTX we expect to be able to compare inner retinal contribution (part of amplitudes that disappears) and outer retinal contribution (remaining parts of amplitudes) of the conventional and global-flash mfERG.

Anesthetic agents and their effect on the mfERG also must be addressed. In porcine mfERG studies, propofol has been considered the drug of choice since it alters the baseline mfERG the least.[2–4] Propofol anesthesia also is preferred in the clinic when mfERG recordings are obtained in patients unable to cooperate. However, the possible existence of a time-dependent effect of propofol on the mfERG could be a problem. Kyhn et al.[6] proposed that a porcine mfERG should be completed within 1 hour of propofol anesthesia. This can be challenging when working with TTX where the postulated time for stabilization of the effect after intraocular injection is 90 minutes.[3,4,10] Consequently, it is necessarily to determine the time-related effect of anesthetics on the mfERG and to reevaluate preferred anesthetic agent.

In this study the relationship between duration of isoflurane and propofol anesthesia and the mfERG will be explored. Furthermore, we will provide methods to minimize time in anesthesia. We expect the findings of this study to be of importance for future clinical and experimental examinations of retinal function.

Methods

The research protocol complied with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Res. and was approved by the Danish Animal Experiments Inspectorate (Permission-number 2012-15-2934-00151). A veterinarian supervised all animal procedures.

Animals

The study included a total of 29 healthy right eyes of female domestic pigs of Danish Landrace /Duroc / Hampshire / Yorkshire breed. The effect of anesthesia on the mfERG amplitude was examined in 12-week-old animals weighing approximately 25 kg. The effect of TTX on the mfERG was examined in animals 14 and 16 weeks old weighing 30 to 45 kg. The sample size in the 3-minute recording study was calculated to be five pigs (correlated measurements in the same animal). The sample size for the anesthesia and TTX study was calculated to be nine pigs in each group (difference between groups). To minimize the number of animals needed, the left eye of 23 pigs was used in other studies: (1) One week before the present study, the left eye of nine pigs had been subjected to increased intraocular pressure for 2 hours followed by an intraocular injection of a neuroprotective substance/vehicle. (2) One to 2 weeks before the present study, the left eye of the other 14 pigs had undergone vitrectomy followed by decaline-induced retinal detachment. The normality of the right eye was evaluated by examining the retina by 20 diopter (D) funduscopy, fundus photography, and histology.

Anesthesia

For 18 hours before anesthesia, animals were fasted but had free access to water. The animals were preanesthetized and the right pupils were dilated as described previously.[13] The pigs were endotracheally intubated and artificially ventilated. Artificial ventilation was supplied with 0.5 l/min 100% oxygen and

2.5 l/min atmospheric air. The stroke volume (10 mL/kg) and respiratory frequency (16/min) were held constant. A total of 20 pigs were anesthetized by the administration of intravenous propofol 15 mg/kg (10 mg/mL; B. Braun Melsungen, Melsungen, Germany). Nine pigs were anesthetized with 2.5% to 3.0% isoflurane (Attane vet; ScanVet Animal Health A/S, Fredensborg, Denmark) administered with 100% oxygen delivered through an endotracheal tube. The pigs were kept hydrated with isotonic saline (9 mg/mL; Fresenius Kabi, Bad Homburg vor der Höhe, Germany) intravenously (IV) and the body temperature was maintained at 38°C to 39°C using a blanket. Heart rate, electrocardiography (EKG), and carbon dioxide and oxygen saturation levels were monitored. In propofol anesthesia, ocular movements were eliminated by intravenous injection of 1 mL of a neuromuscular blocker (Nimbex, 2 mg/mL; Glaxo-SmithKline Pharma A/S, Brøndby, Denmark) every 7 minutes of mfERG recording. Sometimes, an additional 3 mL bolus of propofol was necessary. Ocular stabilization was better with isoflurane anesthesia and the need for Nimbex was on average 1 mL per hour. Before enucleation, fentanyl "Hammeln" (50 μg/mL; Hammeln Pharmaceuticals GmbH, Glouster, UK) 5 μg/kg IV was injected. The anesthetized pigs were euthanized as described previously.[35]

MfERG Recordings

Following 15 minutes of light adaptation mfERGs were obtained in an electrically shielded room using a VERIS Multifocal System with VERIS 6.0.8 software (Electro-Diagnostic Imaging, Inc., Redwood, CA) as described previously.[13] The animal and the respirator were electrically grounded. An infrared (IR) light emitter incorporated in the stimulation camera allowed for continuous fundus monitoring and control of the stability of "fixation."

Two subtypes of 103 unscaled hexagon mfERG were recorded; the conventional one-frame mfERG and the global-flash four-frame mfERG. The conventional 7 minutes 17 seconds of stimulation consisted of an m-sequence exponent of 15, with a frame rate of 75 Hz and 16 samples per frame. The 14 minutes 34 seconds global-flash stimulation consisted of an m-sequence exponent of 14. All other parameters were identical to the conventional recordings. In six propofol anesthetized pigs mfERG was recorded in segments of 3 minutes 38 seconds, with an m-sequence exponent of 14 in conventional recordings and 12 in global-flash recordings. Responses were band-pass filtered outside 10 to 300 Hz. In 20

propofol and nine isoflurane anesthetized pigs global-flash mfERG recordings were obtained approximately 15, 60, and 150 minutes after intubation. Conventional mfERG recordings were obtained after completion of the global-flash recordings (approximately 15 minutes later). The exact topographic localization of the mfERG hexagons was enabled by alignment of the IR-image with the corresponding fundus photo.

Intravitreal Injections

Earlier studies of intraocular injections of TTX in pigs have used an estimated intravitreal volume of 2 ml.[3,4] This is the same as the intravitreal volume of rhesus monkeys,[17] which is a much smaller animal. Therefore, after enucleation of a 3-month-old pig we measured the diameter of the porcine eye and calculated the volume to 4 ml. Furthermore, we injected saline into the vitrectomized enucleated eye and found that it is possible to withdraw 3.4 ml. Consequently, we conducted a pilot study on eight pigs, which showed no statistical difference in the effect of TTX on the mfERG between the 2 and 4 mL groups. For this reason and to compare our results to those of earlier studies, we continued with concentrations corresponding to an intravitreal volume of 2 ml. In 17 propofol and nine isoflurane anesthetized pigs 50 μL 5 μM TTX (Sigma Aldrich Canada Ltd, Oakville, Ontario, Canada) was injected intravitreally with a sterile 29-gauge needle through the pars plana 3 mm posterior to the superior limbus. The retina then was inspected by indirect binocular ophthalmoscopy through a 20 D lens to ensure the absence of bleeding and retinal detachment. Global-flash mfERG was recorded 15 and 90 minutes after injection. Conventional mfERG recordings were obtained after completion of global-flash recordings, which meant approximately 30 and 105 minutes after injection. All injections of TTX were performed in the final anesthesia before the animal was euthanized.

Data Analysis

The VERIS software was used to extract amplitudes and implicit times. We chose to study the conventional and global-flash amplitudes (Fig. 1) as defined previously by Hood et al.,[9] Shimada et al.,[12] and Bearse et al.,[36] for human subjects. It should be noted that the mfERG waveforms varies between species. Consequently, the appearance of the response measured as IC in our porcine model differs from the human IC measured by Shimada et al.[12] The
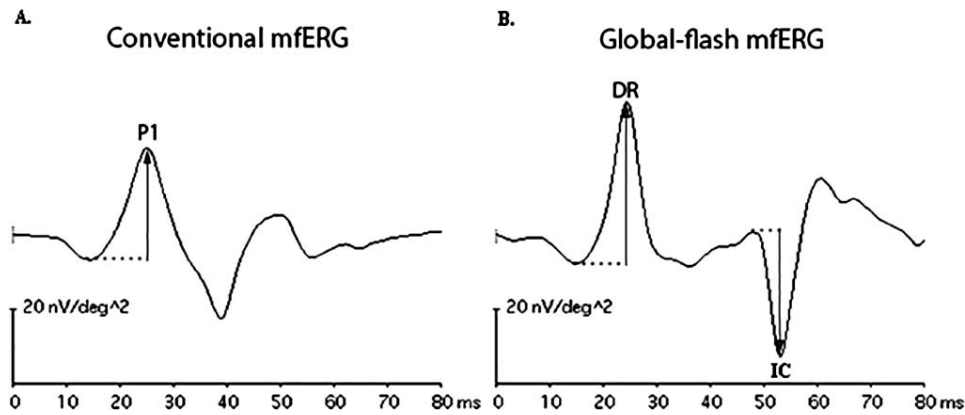
Figure 1. The amplitudes of the mfERG are defined as through-to-peak, but a discrepancy exists regarding which amplitudes to analyze and how they are measured.[2,3,5,8,9,11] Also, variability between species on appearance of the mfERG signal exists. We have chosen to analyze the recordings as described by Hood et al.[9] and Shimada et al.[12] for humans, as these points were easy to identify in all animals and gave no room for subjective interpretation. In agreement with Hood et al.,[9] only the P1 amplitude of the conventional mfERG is analyzed.[8] The global-flash mfERG are analyzed for two amplitudes: the direct component (DR) and the induced component (IC).[11] By default, the VERIS program uses an arbitrary line-0 to calculate the results (not a through-to-peak measurement). To extract the amplitudes correctly, tick the ΔA in the VERIS "custom averages" tab.

appearance of the signal is identical to porcine recordings with the same recording settings, previously published by our group.[14,37,38] The visual streak was identified by alignment of the infrared picture and the fundus image and defined as a band, four hexagons high, stretching horizontally one hexagon above the optic nerve head. The slope of the changes in the mfERG amplitudes with TTX as a function of time in anesthesia was compared to the slope of the amplitude in the anesthesia-only recordings. The individual 3-minute 38-second recordings were merged into three average recordings (7 minutes 17 seconds, 10 minutes 54 seconds, and 14 minutes 32 seconds) using VERIS software. The initial 3-minute recording then were compared to the averaged recordings from the same animal obtained on the same day under the same anesthesia. All data were statistically analyzed with mixed models. For the TTX measurements this was performed with the addition of an interaction between TTX and anesthesia. $P < 0.05$ was considered significant. All statistical analyses were done in SAS Enterprise Guide 7.1® SAS Institute Inc., Cary, NC.

Results

MfERG Recordings

Representative traces of the first and second order kernel of the conventional mfERG and of the global-flash mfERG are presented in Figure 1. The raw mfERG recordings, as well as the topography of the traces, showed a well-defined zone of minimal activity corresponding to the optic nerve head, and a band of larger responses extending horizontally above corresponding to the visual streak.

Anesthesia and TTX Affect the mfERG

Anesthesia affects the major mfERG amplitudes and the induced decline progresses with prolonged duration of anesthesia (Fig. 2). Propofol anesthesia significantly decreases the conventional mfERG amplitudes (Table 1). After 60 minutes of propofol anesthesia, the decrease is estimated to be -4.41 nV/deg^2 for P1 and -1.12 nV/deg^2 for the second order kernel. Isoflurane anesthesia has no significant effect on the conventional mfERG (Table 1). Contrary to the conventional mfERG, the amplitudes of the global-flash mfERG are significantly affected by both isoflurane and propofol anesthesia (Table 1). One hour of propofol anesthesia induces an estimated decrease of -4.82 nV/deg^2 in DR and -4.44 nV/deg^2 in IC. One hour of isoflurane anesthesia induces an estimated decrease of -3.80 nV/deg^2 in DR and -5.58 nV/deg^2 in IC.

There is no significant difference in the effect of TTX between the estimated vitreal volume groups, for example, 2 and 4 mL ($P > 0.41$ for all amplitudes). Therefore, the two groups are combined in the data analysis. From baseline to 15 minutes after TTX injection in propofol anesthesia the slope is significantly different from the slope in pure anesthesia for
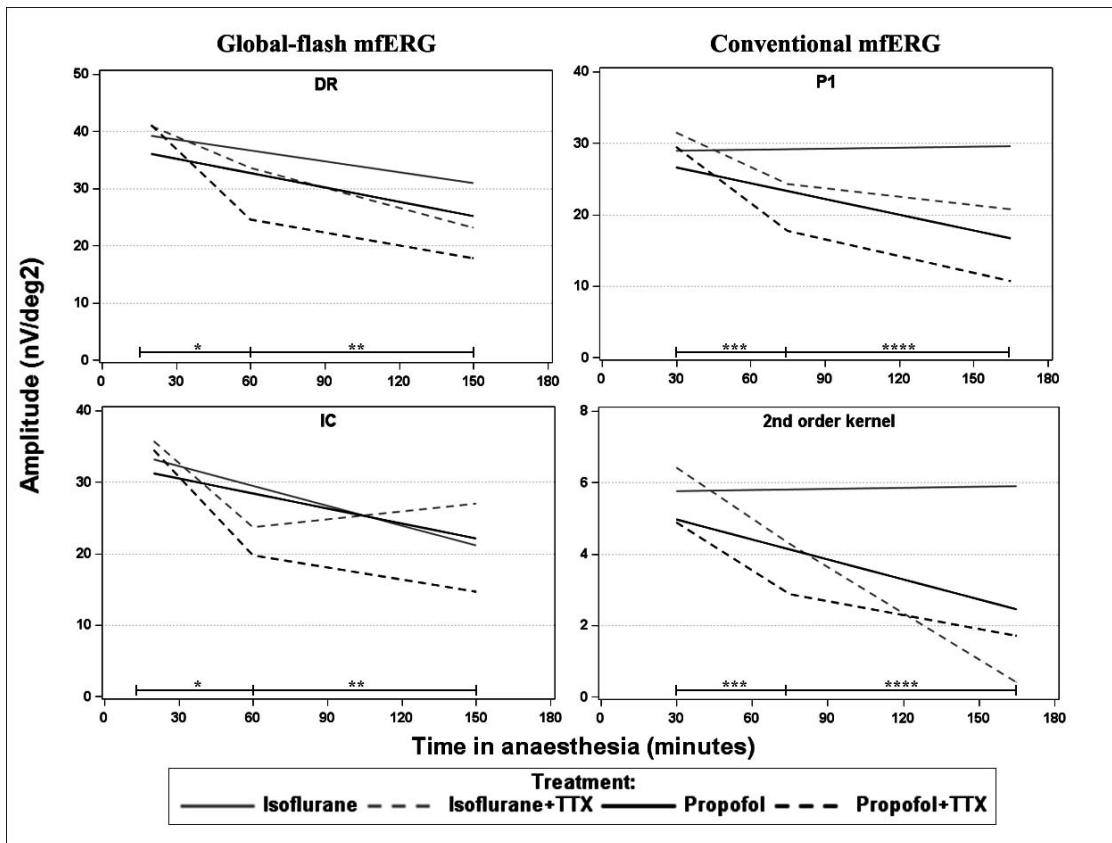
Figure 2. The correlation between time in anesthesia and the major mfERG amplitudes (*solid lines*) and with the addition of intraocular TTX (*dashed lines*). From initiation of anesthesia to initiation of mfERG recording in average 15 minutes is needed to attach the equipment to the animal. Furthermore, conventional recordings were obtained after completion of global-flash recordings and, therefore, are consistently 15 minutes later in anesthesia and in time from TTX injection. *Baseline to 15 minutes after TTX injection (global-flash recording). **15 to 90 minutes after TTX injection (global-flash recording). ***Baseline to 30 minutes after TTX injection (conventional recording). ****30 to 105 minutes after TTX injection (conventional recording). The lines are drawn between the average amplitudes (estimated with mixed model in SAS Enterprise Guide 7.1).

P1, DR, and IC amplitudes (Table 1, Fig. 2). From 15 to 90 minutes after TTX injection in propofol anesthesia the slope is practically identical to the slope in pure anesthesia in the same period (Table 1, Fig. 2). In the isoflurane group a significant effect of TTX was found on conventional amplitudes 15 and 90 minutes after injection (Table 1, Fig. 2). The second order kernel in isoflurane anesthesia had a different pattern than the rest of the amplitudes, showing a time-dependent decline in amplitude after TTX (Fig. 2). There is a slight increase in the slope of IC 15 to 90 minutes after TTX injection in isoflurane anesthesia compared to the slope in pure anesthesia ($P = 0.04$, confidence level [CL], [0.009, 0.28]). The finding is most likely due to random variation as we consider it unlikely that an interaction between isoflurane and TTX could eliminate the effect they each have alone.

The Correlation Between Conventional and Global-Flash mfERG

There is a linear correlation between the conventional P1 and the global-flash DR amplitude, both regarding amplitude and implicit time (Fig. 3). This linear correlation also is found in response to TTX (Fig. 3). The relationship between the second order kernel of the conventional and the IC of the global-flash mfERG is unclear as the amplitude measurements are more dispersed (Fig. 4).

Effect of Reduced Recording Time

Figure 5 shows that the 3-minutes 38-second recordings are practically identical to the prolonged duration recordings. The recorded traces are smooth at all times, which show that they are obtained with a high signal-to-noise ratio (Figs. 5A, 5C). There is a

Table 1. Effect of Anesthesia and Propofol on Major mfERG Amplitudes

	Effect of Anesthesia			Fast Effect of TTX†			Prolonged Effect of TTX‡		
	Slope (nV/deg^2)/ Min	CL	P Value	Slope (nV/deg^2)/ Min	CL	P Value	Slope (nV/deg^2)/ Min	CL	P Value
Propofol amplitude									
P1	−0.08	(−0.13, −0.06)	<0.0001*	−0.19	(−0.33, −0.04)	0.01*	−0.005	(−0.09, 0.08)	0.91
Second order	−0.02	(−0.03, −0.01)	<0.0001*	−0.03	(−0.06, 0.009)	0.14	0.006	(−0.01, 0.03)	0.57
DR	−0.08	(−0.12, −0.04)	0.0002*	−0.23	(−0.39, −0.07)	0.005*	0.02	(−0.06, 0.1)	0.64
IC	−0.07	(−0.12, −0.03)	0.003*	−0.21	(−0.39, −0.03)	0.02*	0.04	(−0.04, 0.13)	0.32
Isoflurane amplitude									
P1	0.003	(−0.04, 0.05)	0.89	−0.16	(−0.30, −0.03)	0.02*	−0.04	(−0.13, 0.04)	0.32
Second order	0.0004	(−0.01, 0.01)	0.94	−0.05	(−0.09, −0.009)	0.02*	−0.04	(−0.07, −0.02)	0.001*
DR	−0.06	(−0.13, 0.003)	0.06	−0.09	(−0.29, 0.11)	0.11	−0.05	(−0.19, 0.08)	0.42
IC	−0.09	(−0.16, −0.03)	0.007*	−0.11	(−0.32, 0.09)	0.28	0.14	(0.009, 0.28)	0.04*

First column shows the time-dependent effect of anesthesia on the mfERG amplitudes for propofol and isoflurane (slope). The second and third columns show the additional effect of TTX in relation to the effect of anesthesia shown in column one (Δ Slope = Slope$_{TTX}$ − Slope$_{anesthesia}$; see Fig. 2). CL, 95% confidence limits; P1 and 2nd order, conventional mfERG amplitudes; DR and IC, global-flash mfERG amplitudes (direct response and induced component).

* $P < 0.05$ (level of significance).

† Global-flash DR and IC recorded 15 minutes after injection, conventional P1 and 2nd order kernel recorded 30 minutes after injection.

‡ Global-flash DR and IC recorded 90 minutes after injection, conventional P1 and 2nd order kernel recorded 105 minutes after injection.

tendency towards a decline in amplitudes with increased recording time (Figs. 5B, 5D). In the conventional recordings this tendency is insignificant, regarding implicit time and amplitude (Figs. 5A, 5B). Compared to the 3-minute recording, there is a significant decline in IC implicit time in recordings 10 minutes or longer and in amplitude in the 14-minute recordings (Figs. 5C, 5D). There is no significant change, either in implicit time or in amplitude, for the DR global-flash amplitude.

Discussion

To the best of our knowledge, this is the first study that shows the time-dependent effect of anesthesia on the retinal function. Proportionate to time in anesthesia, propofol and isoflurane decrease the mfERG-amplitudes. Propofol significantly diminishes all major mfERG amplitudes. Isoflurane primarily affects the global-flash mfERG. The global-flash is thought to contain a higher contribu-

tion from inner retinal cells compared to the conventional mfERG.[5,11,12,20,39–42] This could explain the distinct effect of isoflurane on the global-flash, as the compound blocks 50% of the voltage gated sodium channels found in the inner retina.[5,43,44] Another surprising finding is the stability of the conventional mfERG amplitudes in isoflurane compared to propofol anesthesia. Especially since other studies have found that isoflurane reduces the initial size of mfERG amplitudes compared to propofol.[4,10] One explanation for this difference could be our focus on stability rather than the actual size of the mfERG amplitudes. Another explanation could be an additive effect of propofol and isoflurane in earlier studies. While we obtained all recordings with the same anesthesia on the same day, earlier studies started with propofol and switched to isoflurane.[4,10,45] It is known that the systemic clearance of propofol is low, its half-life is long and the volume of distribution is high.[46] Thus, if propofol was not entirely eliminated the earlier results
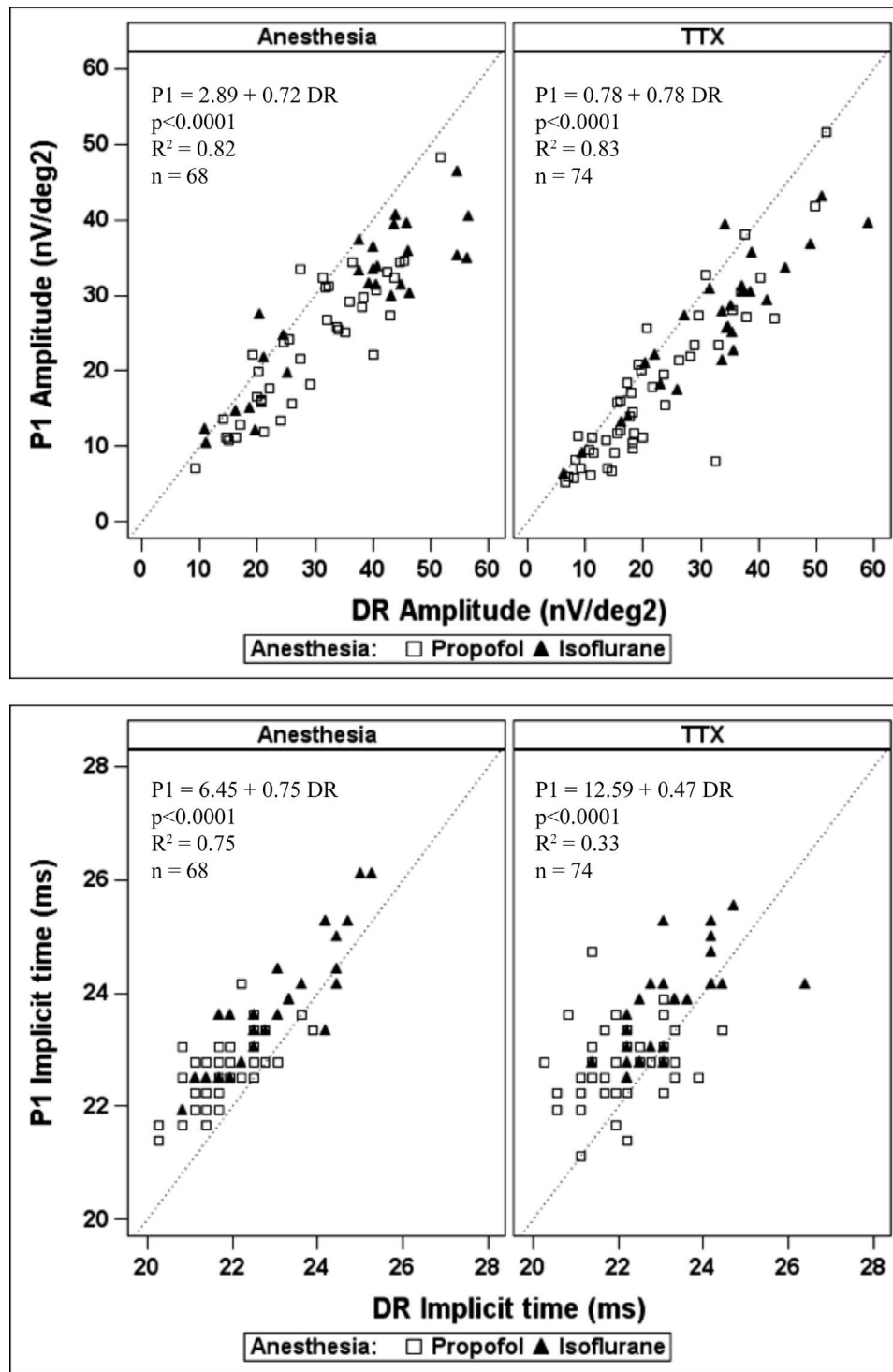
Figure 3. The DR values are plotted against the P1 values for the same pig on the same day under the same anesthesia (agent and duration). Regardless of the anesthetic agent, a linear relationship is seen in the response to anesthesia and in the combination of anesthesia and intraocular TTX. The linear relationship shows that the size of the amplitude (*top*) and the implicit time (*bottom*) for the two amplitudes correlate. The P1 amplitude is lower than the DR. This could be because the animals had been under anesthesia for at least 15 minutes more in conventional recordings as they were obtained consistently after global-flash recordings. The *dashed line* represents the identity line (x = y).
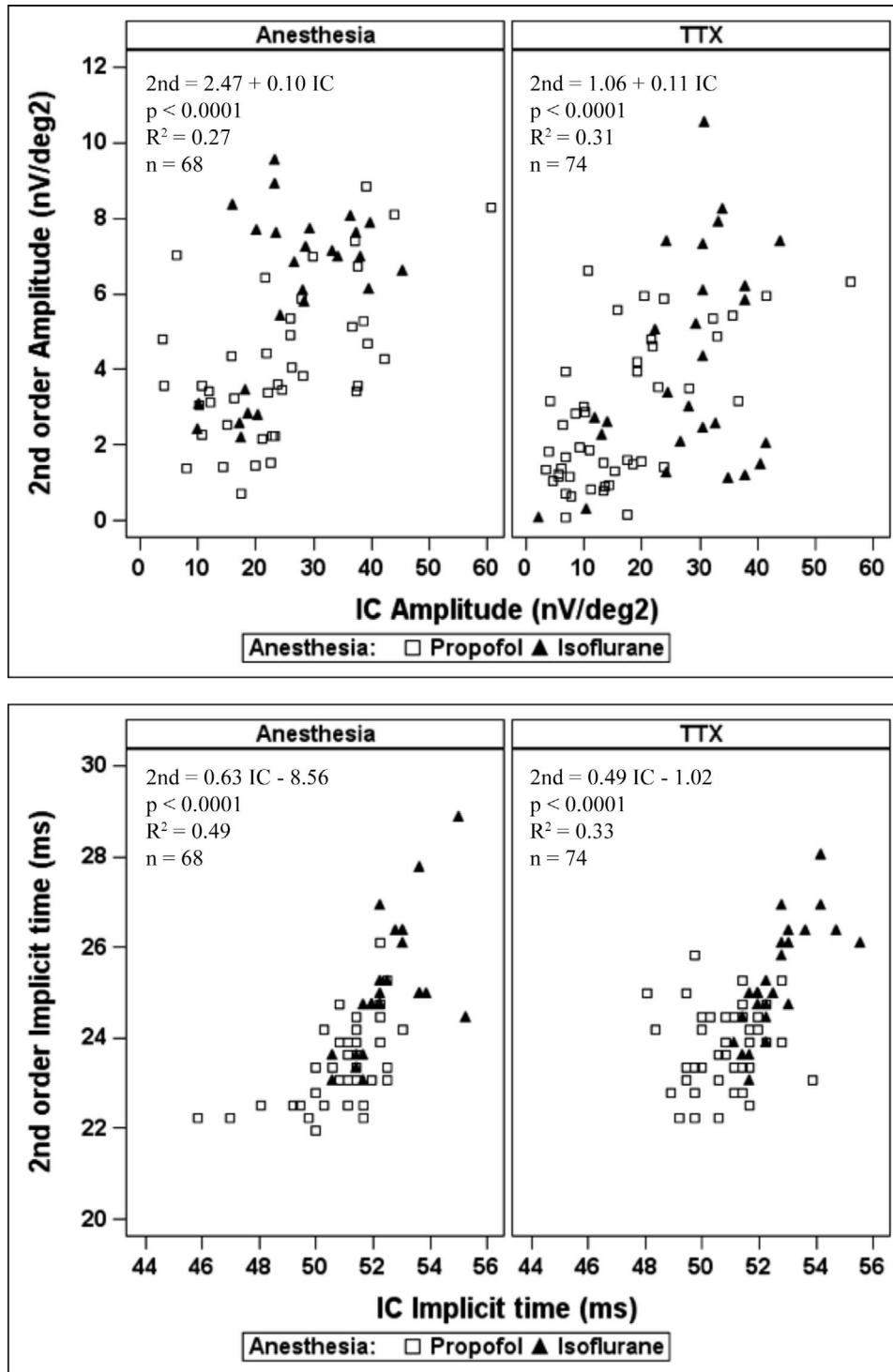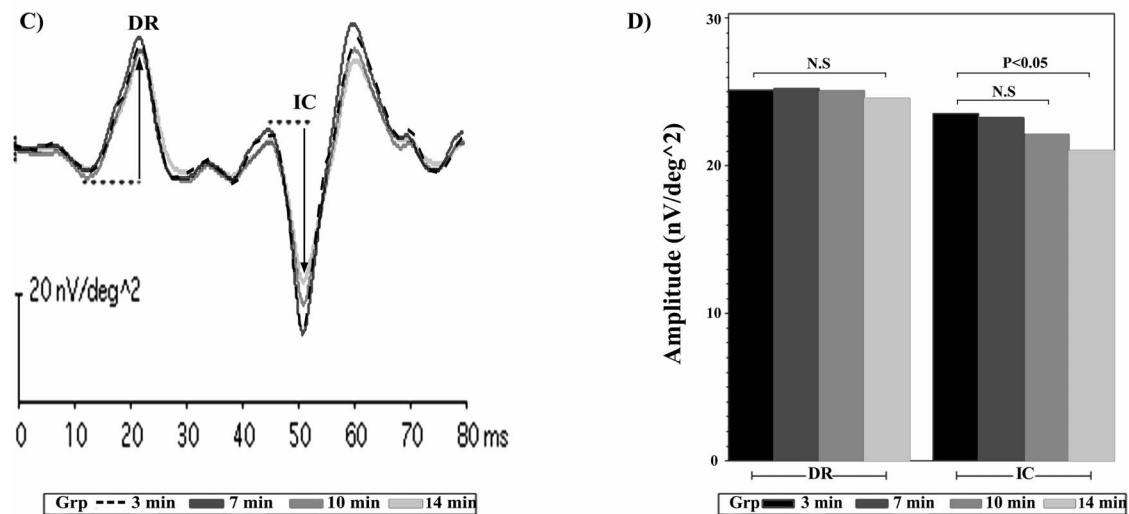
Figure 4. The IC values are plotted against the second order kernel values for the same pig on the same day under the same anesthesia (agent and duration). There is no clear relationship between the two amplitudes. The amplitudes differ in size and implicit time, and are more dispersed compared to the P1 and DR (Fig. 4).
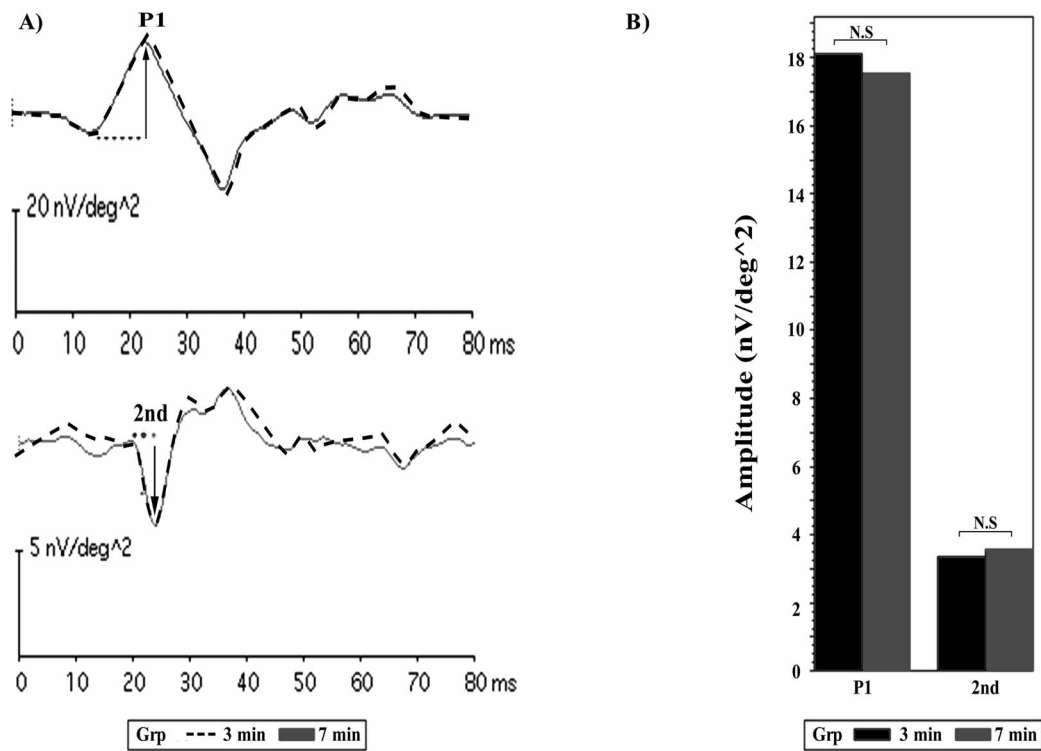
Figure 5. Standard and global-flash mfERG obtained in propofol anesthetized pigs and recorded after 3 minutes 38 seconds (*light gray*), 7 minutes 17 seconds (*medium gray*), 10 minutes 54 seconds (*dark gray*), and 14 minutes 32 seconds (*black*). (A) Conventional mfERG traces for the first order kernel (*top*) and second order kernel (*bottom*). (B) The corresponding average size of the conventional amplitudes. (C) Induced mfERG traces at the four different durations of recording. (D) The corresponding average size of the induced amplitudes; a significant decrease in IC is seen. second, second order; IC, induced component; N.S, nonsignificant. The level of significance was set at $P < 0.05$.

favoring propofol might be explained by an additive effect of the two anesthetic agents. Another possible explanation is the duration of anesthesia, as isoflurane recordings have been consistently obtained after the completion of propofol recordings.[4,10,45]

Potentially, the time-related effect of anesthesia on the retinal function could be caused by the instability of vital parameters. Earlier studies have suggested that the ERG is affected by respiratory and ventilatory changes.[7] In our study, all animals were intubated and the heart rate, oxygen saturation levels and body temperature were kept constant. For this reason, we do believe our findings are induced by the respective anesthetic agents. Since the understanding of how anesthetics affect the retina at a molecular level is limited, it is difficult to understand the time-dependent effect of propofol and isoflurane on retinal function. It is known that the primary target of propofol is the $GABA_A$ receptor found in the bipolar, amacrine, horizontal, and retinal ganglion cells while isoflurane show little selectivity for molecular targets and act on several receptors.[47–49] A more thorough discussion of the molecular targets in the retina is beyond the scope of this article and we refer to te study of Kim et al.[2]

Regardless of the type of anesthesia, we did not find high-frequency oscillations in our mfERG recordings. Such high-frequency oscillations are thought to represent retinal ganglion cell activity.[3] Earlier studies have shown that these oscillations are profoundly affected by anesthetics.[5,45,50] In the porcine model, high frequency oscillations can be induced by ketamine anesthesia in combination with TTX.[3] These results could not be reproduced with isoflurane anesthesia.[3] It is difficult to tell whether these porcine oscillations are the product of an interaction between ketamine and TTX or represent an inner retinal signal in itself that is amplified by TTX. However, we can conclude that high-frequency oscillations are absent in porcine mfERG obtained purely under ketamine,[3] propofol, or isoflurane anesthesia.

Effect of TTX and Comparability between Conventional and Global-Flash mfERG

Our study provided new knowledge regarding the speed of action after intraocular injection of TTX. In isoflurane, TTX significantly decreases the conventional P1 and second order kernel mfERG amplitudes. This effect of TTX on the conventional mfERG is in accordance with earlier published work.[4,5,10,15,17,30] The new in our results is that we have examined and found the effect of TTX within 30 minutes after intraocular injection. The fast effect of TTX also is found in propofol, where TTX within 15 to 30 minutes after injection significantly decreases the P1, DR, and IC amplitudes. In the period 15 to 90 (30–105) minutes after TTX injection there is no significant additional effect of TTX compared to the effect of pure propofol anesthesia (Fig. 2).

It has been common in earlier studies to allow stabilization of effect for over 90 minutes after intraocular injection of TTX.[3,4,10] A significant effect of TTX on the IC has been found in the porcine model 90 minutes after intraocular injection.[10] This decrease in amplitude is correct, but in the earlier study the effect was attributed to TTX alone. At 15 minutes after intraocular injection of TTX in propofol anesthesia, we found that the TTX primarily causes the decrease in IC amplitude (Fig. 2). At 90 minutes after the injection a major part of the decrease in the amplitude can be attributed to the anesthesia and not only the TTX (Fig. 2). It is not known to what extend this effect is present in humans as well.

Our results showed that the anesthesia in itself decreases the mfERG amplitudes in a time-dependent manner. Therefore, with longer stabilization for full effect of TTX the results are contaminated by the effect of prolonged anesthesia. We propose that pilot studies are performed in future investigations to estimate optimal time from intraocular injection to effect of a drug.

Our results showed a close relationship between the conventional P1 and the global-flash DR amplitude and suggested that they represent the same retinal signal. We did not find the same comparability between the conventional second order kernel and the global-flash IC.

Three-Minute mfERG Recording is Sufficient

We find that a 3-minute mfERG recording, obtained in an electrically shielded lab, is adequate in anesthetized animals. Under these conditions the signal-to-noise ratio is high and prolonged recordings to filter noise are unnecessary. Previously, duration of 8 to 16 minutes has been recommended to increase the signal-to-noise ratio.[9,29] Contrary to this, we found that prolonged recordings may lower the quality of the mfERG signal. There is a significant decrease in the global-flash IC amplitude after 14 minutes compared to 3 minutes of recording. Exhaustion of retinal cells by repeated light stimulation might

explain the decrease in the amplitude with prolonged recording. Another explanation might be the increased duration of anesthesia in prolonged recordings. A relevant concern when the m-sequence exponent is reduced is the risk of kernel overlap and thereby erroneous localization of response contributions. The VERIS system is designed to minimize this risk by choosing the best m-sequence of the length you specify. Even though stimulation events in the conventional protocol are only 13.3 ms apart, a reduction in m-sequence exponent from 15 to 14 should not be a problem. The chance of kernel overlap in the global-flash protocol is much smaller as the use of four frames keeps the stimulation events 53 ms apart. Additionally, almost all the response power in the global-flash stimulation is found in the first order kernel. Therefore, the risk of kernel overlap in the global-flash stimulation is low and the m-sequence exponent can be chosen based on the signal-to-noise ratio you want to achieve. Our finding that a shorter duration of mfERG recording provided a stronger signal might also be applicable to human mfERG recordings in general anesthesia.

Considerations for the Future Choice of Anesthetic Compound

We found a greater need for muscle-relaxants (Nimbex) in the propofol group compared to the isoflurane group. This corresponded to earlier findings.[1–4] It could be speculated that the Nimbex contributed to the difference between propofol and isoflurane in conventional mfERG recordings. Nimbex is a cistracurium besylate which acts on nicotinic acetylcholine (cholinergic) receptors and has an elimination half-life of 22 minutes. Nicotinic acetylcholine receptors are found in retinal cells in several species.[51–56] The presence of the receptors in the retina implies that Nimbex might have some influence on the mfERG. It is known that isoflurane prolongs the clinically effective duration of action of Nimbex and decrease the average infusion rate requirement. A prolonged effect of Nimbex in the isoflurane group could explain the lesser need for the muscle-relaxant to achieve ocular stability. It also could indicate that the effect of Nimbex in the two groups might have been similar, even though the administrated dose differed. Furthermore, if a cumulative effect of Nimbex existed, we also would have expected it to improve ocular stability with increased amounts. However, the need for Nimbex to achieve ocular stability in propofol recordings remained unaffected

of the number of repeated injections. For this reason we find it unlikely that Nimbex contributed to the time-dependent decrease seen in conventional mfERG recordings in propofol compared to in isoflurane. Another explanation might be accumulation of propofol, since infusion rates remained stable.[57] Accumulation of propofol also would explain our recovery time of 1 to 3 hours after termination of prolonged intravenous infusion. The equivalent recovery time for isoflurane was 30 minutes, which is in accordance with that isoflurane, as a volatile gas, is not expected to accumulate.[57] Therefore, in future mfERG studies using propofol, we recommend that the infusion rate is adjusted to the duration of anesthesia.

Another anesthetic drug that has been compared to isoflurane in earlier porcine mfERG experiments is ketamine. The measurable effect of optic nerve sectioning has been shown to disappear when anesthesia is changed from ketamine to isoflurane.[3] From this perspective it could be argued that ketamine is superior for porcine mfERG studies. Despite this, we chose to exclude ketamine in the present study. We previously have tried to obtain porcine mfERG recordings under ketamine anesthesia, but experienced great difficulties in obtaining a sufficient level of anesthesia (unpublished data). Furthermore, with this anesthetic, we have experienced considerable variability in mfERG results and problems with maintaining ocular stability (unpublished data). This is in agreement with the experience of other groups using ketamine anesthesia in pigs.[58] For this reason, we consider isoflurane and propofol to be the most suitable anesthetic agents for porcine mfERG studies.

Conclusion

Anesthesia is difficult to avoid in animal mfERG studies and the hope of finding an anesthetic agent without an effect on the mfERG might be small. Consequently, rather than trying to eliminate the changes induced by the anesthetic agent we need to control them. It is accepted that care must be taken when comparing mfERG data collected under different anesthetic agents.[59] Our results further emphasized that care should be taken when recordings obtained with variable duration of anesthesia are compared. In conclusion, we recommend that duration of anesthesia in mfERG recordings should be kept at a minimum.

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