Published in final edited form as:

Doc Ophthalmol. 2012 February ; 124(1): 59–72. doi:10.1007/s10633-011-9306-x.

The effect of pentobarbital sodium and propofol anesthesia on multifocal electroretinograms in rhesus macaques

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Abstract

We compared the suitability of pentobarbital sodium (PB) and propofol (PF) anesthetics for multifocal electroretinograms (mfERGs) in rhesus macaques. mfERGs were collected from 4 ocularly normal rhesus macaques. All animals were pre-anesthetized with intramuscular ketamine (10-15 mg/kg). Intravenous PB induction/maintenance levels were 15 mg/kg/2-10 mg/kg, and for PF, 2-5 mg/kg/6-24 mg/kg/h. There were 3 testing sessions with PB anesthesia and 5-7 testing sessions with PF anesthesia. All PB sessions were carried out before PF. First-order (K1) and second-order (first slice) kernels (K2.1) response density amplitude (RDA), implicit time (IT), and root mean square signal-to-noise ratios (RMS SNR) of the low-frequency (LFC) and high-frequency (HFC) components were evaluated. The use of PF or PB anesthesia resulted in robust, replicable mfERGs in rhesus macaques; however, RMS SNR of K1 LFC in ring and quadrant analyses was significantly larger for PF than for PB. Additionally, K1 RDA under PF was significantly larger than under PB for N1, P1 and P2 components (ring and quadrant), and for N2 (quadrant). PF IT was significantly prolonged (<1 ms) relative to PB IT for N1, P1 (ring) and N1 (quadrant) while PB IT was significantly prolonged (0.8-4.2 ms) relative to PF IT for N2 and P2 (ring and quadrant). K1 HFC and K2.1 LFC did not differ significantly between PB and PF in the ring or quadrant analyses. The response differences found with PB and PF anesthesia likely arise from variable relative effects of the anesthetics on retinal γ -aminobutyric acid (GABA_A) receptors, and in part, on glycine and on glutamate receptors. Given the advantages of a stable anesthetic plane with continuous intravenous infusion and a smoother, more rapid recovery, PF is an appealing alternative for mfERG testing in rhesus macaques.

Keywords

multifocal electroretinogram; rhesus macaque; pentobarbital sodium; propofol; root mean square; signal-to-noise ratio

INTRODUCTION

Multifocal electroretinography testing in nonhuman primates has been carried out with varied anesthetic regimens, e.g., ketamine and xylazine [1-4], ketamine with neuromuscular blocker intravenous infusion [5-6], ketamine or ketamine and xylazine and isoflurane with extraocular muscle blockade [7], and propofol with periodic injections of a neuromuscular blocker [8-10]. Some of the studies [5-6] [8-10] have incorporated the use of a systemic neuromuscular blocker. Our laboratory has used pentobarbital sodium and isoflurane anesthesia without a paralytic agent in previous investigations of the multifocal electroretinogram (mfERG) [11-14] in rhesus and cynomolgus macaques.

The effects on retinal responses may differ when varying anesthetic agents are administered during multifocal electroretinographic recordings. Duration of anesthesia, route or method of delivery, class and concentration of anesthetic compound have been found to influence systemic effects and also to influence locally retinal function measures [7] [15-18]. In an earlier study [19], we achieved good ocular stability using a continuous intravenous infusion of propofol without a paralytic agent in acquiring simultaneous pattern ERG (PERG) and visually evoked cortical potentials (VEPs) with the sweep-VEP technique. Here, we verify the suitability of propofol in collecting mfERGs and directly compare the relative influences of pentobarbital sodium and propofol anesthesia on the mfERG within the same subset of rhesus macaques.

METHODS

All of the experimental methods and techniques adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by our institution's animal care and use committee. The general experimental procedures have been described previously [11-12]. A brief description of the specific study methods and any deviations are presented below.

Subject animals

Four adult female rhesus (*Macaca mulatta*) macaques (weight/age ranges: 5.3 –7.8 kg/9-12 years) were used in the data collection for this study. The animals were experimentally naïve to visual function testing and ophthalmologic procedures. All animals had been examined with slit lamp and indirect ophthalmoscopy at the initiation of the study, and were found to be ocularly normal.

Anesthesia

Each animal was pre-anesthetized with ketamine [10-15 mg/kg, intramuscular (IM)], and a vein was catheterized for either intravenous (IV) injections of pentobarbital sodium (induction: 15 mg/kg, maintenance: 2-10 mg/kg) or continuous IV infusion of propofol (induction: 2-5 mg/kg maintenance rate: 6-24 mg/kg/h). Because of pentobarbital's specific anesthetic effects (non-uniform influence and lack of an acceptable average dosage), IV injection (and not a continuous IV infusion as permissible with propofol) of pentobarbital sodium was the preferred method of administration, but may not maintain a consistent level of anesthesia. Generally, the amount of ketamine (for pre-anesthesia) injected was sufficient to allow the animal to be transported from the home cage/room into the electrophysiologic testing facility, to be weighed, to have intraocular pressures measured, and to have an intravenous catheter situated. In this manner, ketamine anesthesia effects and influences were minimized before the induction with pentobarbital sodium or propofol. A mean of between 13 to 34 minutes for pentobarbital sodium and between 17 to 31 minutes for propofol occurred from the initiation of pre-anesthesia with ketamine to induction with the respective general anesthetic. For both pentobarbital sodium and propofol, the equivalent of stage III plane 2 of surgical anesthesia was achieved. The animal was immobile, non-responsive to tactile stimulation, and ocular position was centrally located with cessation of the corneal reflex. For pentobarbital sodium, induction of general anesthesia was accomplished with an intravenous dose of 15 mg/kg. If a supplement was required (e.g., ocular movements noted during alignment of the second eye tested before mfERG recording), then a 2-10 mg/kg IV injection was administered. The amount of supplement was dependent upon the time point in the recording at which supplementation was necessitated. Unlike with propofol anesthesia, a supplemental injection of pentobarbital sodium during stimulus presentation (or actual recording) of the multifocal electroretinogram was uncommon. After initial mfERG recordings with propofol (during

which optimization of anesthetic techniques were carried out, e.g., a lower rate (6-10 mg/kg/h) was administered initially and then followed by successively higher rates to effect), a standard technique for achieving general anesthesia was applied to each animal to obtain immobility, ocular stability, and consistent vitals measures. Post induction of general anesthesia with a bolus of 2-3 mL (duration: 30-45 s) of intravenous propofol, the animal received a continuous IV propofol maintenance infusion rate of 24 mg/kg/h. During baseline mfERG recordings, the maintenance infusion rate was tolerated well by all four animals. On occasion, after establishment of the maintenance rate, a bolus (1-3 mL delivered in 15-45 s) of propofol was administered to control ocular drift and return the eye to central fixation (verification of central ocular fixation was confirmed with the reversing ophthalmoscope before proceeding with response data collection). During administration of a bolus, the animal's heart rate, SpO₂, and respiration rate were monitored carefully. Heart rate, percent oxygen saturation (SpO₂), respiration rate, and rectal temperature were monitored every 0.25 h and remained within the limits established by our laboratory's Animal Care and Use protocol. These parameters did not differ between pentobarbital sodium and propofol for any of the animals. The heart rate (stable and regular without large variations [< 10 -20 beats per minute variability from a stabilized rate] and SpO₂ ($\geq 90\%$) were monitored with a pulse oximeter. Body temperature was maintained between 37°C and 39°C. Fluid supplementation was provided (up to 10 mL/kg/h) in a subcutaneous bolus post procedurally. Blood pressure and pCO₂ levels were not routinely monitored. Pupils were fully dilated and accommodation fixed with 1% tropicamide and 2.5% phenylephrine hydrochloride ophthalmic solutions. Eyelids were held open with a lid speculum. ERG-jetTM corneal contact lens electrodes were applied bilaterally to the corneal surfaces with a drop of 2.5% hydroxypropyl methylcellulose ophthalmic solution. Each animal was tested on 3 different days (separated by at least one week between tests) with the use of pentobarbital sodium anesthetic. With propofol, each animal was tested on 5 to 7 different days (separated by at least one week between tests). A time period of 5 months separated the pentobarbital sodium and propofol testing sessions, with all of the pentobarbital sodium sessions carried out before the propofol sessions. Typical recording duration for each animal ranged between 1.0 to 1.5 hours.

Recording procedures

The active ERG-jetTM corneal electrode was referenced to a subdermal needle electrode that was situated at the ipsilateral outer canthus. A subdermal needle electrode inserted in the lower arm served as the ground. Impedances were ≤ 5 kilohms and equivalent for the active, indifferent and ground electrodes used. The mfERG responses were collected with the use of VERIS ScienceTM 4 (Electro-Diagnostic Imaging, Inc., Redwood City, CA). Signal amplification was 20,000 with band pass filtering from 3 Hz to 300 Hz. Testing was monocular with the nontested eye occluded with an opaque patch. The first eye tested on the initial day of mfERG recordings was determined randomly, and then subsequently, the order of first eye tested was counterbalanced.

Visual stimulation procedures

241 equal-sized hexagonal elements were displayed on a 21" monochrome monitor at a frame rate of 75.0 Hz (13.3 ms per frame). The viewing distance was 20 cm, and the diameter of the stimulus array subtended approximately 80 deg of the central visual field. The eyes were refracted for the testing distance. For an ocularly normal animal, a +5D trial lens corrected optimally at the viewing distance of our mfERG testing procedures. The trial lens was situated at approximately 7 mm anterior to the vertex (surface aspect of the cornea), and thus was approximately 13 mm from the posterior nodal point (rear of crystalline lens) of the animal's eye. A reversing ophthalmoscope affixed with a corner cube prism was used to align the fovea with the center of the stimulus display, and to verify the particular refractive status of the animal. Maximum and minimum luminances of the display

were 200 cd/m² and ~1 cd/m². Mean luminance was ~ 100 cd/m². Stimulus elements were alternated according to a binary maximum-length sequence of 2¹⁵-1 m-steps. Sampling rate of the signal was 1200 Hz (0.83 ms).

After alignment and initiation of mfERG, the trace recording on the operator's monitor and the animal's eye were observed carefully for any perturbations or indication of ocular movements. The approximately 7-minute recording period was subdivided into 4 equal temporal segments that permitted opportunities to examine ocular position (and carry out realignment procedures after anesthetic supplementation) before re-initiation of a recording. Two of the 4 animals manifested a tendency to exhibit ocular movements with propofol anesthesia. In animals with observed ocular movements or ocular drift, a supplemental bolus (1-3 mL infused over about 15 s to 45 s, followed by the immediate resumption of the maintenance rate) was generally sufficient to eliminate these movements further during mfERG recording. With pentobarbital sodium anesthesia, ocular drift was not noted ordinarily during mfERG recording; if ocular movements did occur, they were typically small drifts from central fixation observed during use of the reversing direct ophthalmoscopic device for foveal alignment with the stimulus array. Thus, for pentobarbital sodium generally, a mid point (i.e., in the period between the testing of the two eyes in a session) supplementation (IV injection) was sufficient to prevent ocular movements during mfERG recording of the second eye.

mfERG Response Analysis

Response density amplitudes (RDAs or amplitudes) and implicit times (ITs) were derived from grouped ring averages or quadrants of the first-order kernel (K1) and second-order kernel, first slice (K2.1) trace arrays. Each trace array was grouped into 6 rings (Figure 1, inset) or quadrants (Figure 5, inset), and then the processed data (from the VERIS Science 4.1 software) were exported to a task-specific MATLAB routine that was applied in the derivation of quantified waveform components. Specifically, a zero-phase delay filter was used to remove the frequencies > 80 Hz in the raw data waveforms. Then, an automatic peak-finding routine was used to derive the RDAs and ITs of the K1 (N1, P1, N2, and P2) and K2.1 (p1 [initial prominent positive peak], n2 [negative deflection subsequent to the p1 positive peak], and p2 [positive peak subsequent to the n2 negative deflection]) primary wave components (refer to Figures 1 and 2 for peak locations), which were output to a data base and compared in the statistical analyses. The automatically scored waveforms were examined individually, and any peak components were adjusted manually (infrequent occurrence) when a designated peak component was clearly incorrect. Root mean square (RMS) was low pass (< 80 Hz) filtered for the response amplitudes (LFC) and high pass (> 80 Hz) filtered for oscillatory potentials (HFC). For RMS of K1 and K2.1 LFC and K1 HFC (Figure 3), signal and noise time segments were analyzed between 10 ms and 180 ms and between 700 ms and 870 ms, respectively. The specific noise segment (relatively quiescent responsiveness) was selected to ensure that signal effects would not influence this subsequent temporal region of the response, and likewise that noise effects would not influence the signal segment.

Statistical Analysis

A repeated measures ANOVA split plot design tested the effect of anesthesia, test eye, and retinal eccentricity (ring) and retinal quadrant on each of the primary wave components, and were individually analyzed for the K1 and K2.1 RDA and IT measures, and root mean square signal-to-noise ratios (RMS SNR) of K1 and K2.1 LFC and K1 HFC. The level of statistical significance adopted was $p < 0.05$. The Greenhouse-Geisser epsilon adjustment was applied to the univariate repeated measures statistical tests when the sphericity assumption was violated.

Typically, initiation of a new anesthetic regimen requires a period within which familiarity with the anesthetic (in this study, propofol) is optimized for the experimental procedure and within each of the animals tested. To ensure relative equivalency of the technique for each of the anesthetics administered and optimization of the anesthetic requirements of individual animals, and thus, that mfERG responses (and not technique differences) were evaluated, the last three test sessions with propofol anesthesia of each animal were included within the statistical comparisons.

RESULTS

K1 RDA (amplitude) and IT

Ring (retinal eccentricity) analysis—For N1 ($p<0.0005$), P1 ($p<0.0005$), and P2 ($p=0.028$) waveform components, K1 RDAs were significantly larger with propofol (PF) anesthesia compared with pentobarbital sodium (PB) anesthesia (Figure 1). K1 ITs for N1 and P1 differed from that for N2 and P2 waveform components in that under PF anesthesia, ITs were more prolonged than under PB anesthesia for N1 ($p<0.0005$, 0.5-ms prolongation) and P1 ($p=0.003$, 0.7-ms prolongation). The effect of anesthesia on IT prolongation was the obverse for N2 ($p=0.001$, 0.8-ms prolongation) and P2 ($p=0.001$, 4.2-ms prolongation) with greater prolongation for PB than for PF.

K2.1 RDA and IT

Ring analysis—Significant differences in K2.1 RDAs for p1 ($p<0.0005$) and n2 ($p=0.034$) were observed, with PB anesthesia resulting in higher amplitudes (p1: 28% and n2: 13%) than with PF anesthesia (Figure 2). IT was more prolonged ($p<0.0005$) for p1, n2 and p2 while under PB than under PF anesthesia. The prolongation varied between 1.754 ms to 2.112 ms ($\leq 9\%$) with PB. For n2 and p2 waveform components, IT was more prolonged for PB than PF anesthesia in the central (rings 1-3) as compared to the more peripheral retina (rings 4-6).

RMS SNR Amplitude

Ring analysis—RMS SNR of the K1 LFC were greater with PF than with PB anesthesia ($p<0.0005$) (Figure 4a). For the central 5.3 degrees of retina, RMS SNR disparity was lesser ($p=0.001$) than for more peripheral retinal eccentricities. No significant differences were observed for either K1 HFC (Figure 4b) or for K2.1 LFC (Figure 4c). Consistent noise time segment differences were not observed across retinal eccentricity in comparisons of mfERG responsiveness between pentobarbital sodium and propofol anesthesia.

K1 RDA (amplitude) and IT

Quadrant analysis—Similar to the ring analysis, K1 RDAs in the quadrant analysis were significantly larger (N1, P1, N2, and P2: $p\leq 0.001$) when collected under PF anesthesia than under PB anesthesia (Figure 5). For IT, N1 was more prolonged for PF than PB anesthesia ($p<0.0005$); however, for N2 and P2 waveform components, ITs were more prolonged ($p<0.0005$) for PB than PF anesthesia. Naso-temporal differences were significant for K1 RDA for only N2 both for PB ($p=0.043$) and PF ($p=0.037$), with mean differences less than 10% of the actual mean amplitude values. For K1 IT, significant naso-temporal differences were found for N1, P1, and N2 for PB and PF; the mean differences ranged between 2% and 5% of the actual mean temporal values.

K2.1 RDA and IT

Quadrant analysis—Also similar to the ring analysis for K2.1 RDA, responses collected under PB anesthesia were significantly larger than under PF anesthesia for p1 ($p<0.0005$,

~51% larger) and n2 ($p < 0.0005$, ~19% larger). The obverse was observed for p2 with significantly larger amplitude ($p = 0.026$, ~10% larger) for PF than for PB anesthesia (Figure 6). IT was more prolonged ($p < 0.0005$) for p1 (2 ms, 11% IT disparity), n2 (1.3 ms, 4.5% IT disparity) and p2 (2 ms, 4.8% IT disparity) waveform components collected under PB than PF anesthesia. A significant naso-temporal difference of K2.1 RDA for PB and PF in n2 was $\leq 0.5 \text{ nV/deg}^2$. K2.1 IT naso-temporal differences were significant for p1, n2, and p2 (for PB) with mean differences ranging largely between 1% and 4% of the actual mean temporal values.

RMS SNR Amplitude

Quadrant analysis—K1 LFC (RMS SNR) were greater with PF than with PB anesthesia ($p < 0.0005$) (Figure 7a), which was also observed in the ring analysis. Significant differences were not found for either K1 HFC (Figure 7b) or K2.1 LFC (Figure 7c). For both PB and PF, significant naso-temporal differences were found only for K1 HFC ($p < 0.0005$). As for the ring analysis, consistent noise time segment differences were not observed across quadrants in comparisons of mfERG responsiveness between pentobarbital sodium and propofol anesthesia.

DISCUSSION

Ocular movements must be eliminated to ensure successful multifocal electroretinography recordings. In early investigations within our laboratory, an IM injectable combination of ketamine/xylazine without paralysis did not provide sufficient ocular stabilization for acceptable recordings, although such an anesthetic combination has been applied successfully by other investigators [1-4]). In accompanying studies, the use of pentobarbital sodium anesthesia with mfERG and mfVEP recordings has resulted in a stable animal preparation and reproducible and robust functional results [11-12] [14] [20]. We also have anesthetized with a volatile inhalant (isoflurane) successfully [13] during electrophysiological recordings; however, with this anesthetic, experimental studies cannot be prolonged to avoid potential depression of the mfERG response [7]. Additionally, the evoked response potential (ERP) and the visually-evoked response (VER) collected under isoflurane anesthesia have been shown to become depressed or virtually eliminated and the latency measures and the character of the evoked response markedly altered during general anesthesia with volatile anesthetics [15] [21]). Under propofol anesthesia alone, achievement of stable ocular position was accomplished in our earlier sweep-VEP study in rhesus macaques [19].

In the present study, anesthesia with both pentobarbital sodium (PB) and propofol (PF) enabled stable ocular fixation and robust, reproducible mfERGs. Primarily, effects of anesthetic regimen were observed in the K1 mfERG measures of both the ring (retinal eccentricity) and quadrant analyses. Amplitude of the early- and late-wave components (N1, P1, and P2 for ring; N1, P1, N2, and P2 for quadrant) of the K1 mfERG and of the K1 LFC (RMS SNR) with PF anesthesia were significantly enhanced when compared to animals anesthetized with PB. K1 IT differed for the early-wave (N1 and P1) and late-wave (N2 and P2) components with PF anesthesia prolonging early-wave and PB prolonging late-wave components both for ring and quadrant analyses; however, except for the P2 wave component, the delay in IT (though statistically significant) was $\leq 0.8 \text{ ms}$, and so probably not biologically significant. K2.1 LFC and K1 HFC (RMS SNR) measures for ring and quadrant analyses did not differ between the two anesthetic regimens; however, disparities in amplitude (significantly larger for PB in p1 and n2) and/or IT (significantly more prolonged for PB in p1, n2, and p2) of the K2.1 mfERG were observed. If the notions that K1 and K2.1 mfERG responses reflect largely outer retinal and inner retinal activity,

respectively, then the K2.1 in combination with the K1 results suggest that specific anesthetic influence on the outer and inner retina, may depend somewhat on the particular agent used.

Recent electrophysiologic studies [17] [22-24] have compared the effects of varied general anesthetics on mammalian focal ERG, PERG, oscillatory potentials (OPs), scotopic threshold response (STR), and scotopic and photopic ERGs. In those studies in which barbiturate anesthesia was administered [17] [22], electrophysiologic responses were depressed relative to other anesthetics or anesthetic combinations administered (ketamine/xylazine, alphaxalone/alphadolone, halothane, urethane or telazol), but PF was not administered in either study.

More commonly, investigations of PB or PF effects on visual function have found differences in neuronal responses subserving purported outer and inner retinal function. Kapousta-Bruneau [25] reported suppression by sodium pentobarbital of the scotopic threshold response and oscillatory potentials in the isolated rat retina, but not on photoreceptor function. PF has been shown to enhance electrophysiological responsiveness in canines [26]. A strong reversible increase of scotopic b-wave and of a-wave peak amplitude was noted after an increase (50%) in propofol infusion rate. Ver Hoeve, Danilov, Kim and Spear [19] showed little effect (a slight decrease) on pattern ERG and VEP when PF rate was increased 100%. Relatedly, when anesthetic agents (including ketamine/xylazine, PF, PB, urethane, and isoflurane) were compared on the ERP of the rat whisker sensory system [21] ERP was highest and the latency slowest under PF anesthesia. PB anesthesia resulted in a lower and less prolonged (~ 35%) ERP peak amplitude when compared to propofol anesthesia. Both ERP findings were generally consistent with our K1 mfERG results.

Studies in the retina and central nervous system have shown that signal transduction depends not only on the identity of the transmitter substance(s) but also upon the receptors at which it binds [27]. GABA (the principal inhibitory neurotransmitter in the brain) and glycine are the primary fast inhibitory neurotransmitters in the central nervous system and retina [16] [27-33] acting on GABA_A and glycine receptors, respectively, which are Cys-loop pentameric ligand-gated ion channels that are chloride ion-selective [16] [18] [29] [34-36]. Although the effects of general anesthetics at the molecular level are not fully understood, the primary molecular target of intravenous anesthetics such as pentobarbital and propofol are the GABA_A receptors, which are found abundantly in outer and inner retinal neurons [28] [31] [35] [37]. Based upon the clinical features of glycine receptors, they appear to be the primary molecular targets for the volatile (halogenated ether) anesthetics [29] [33] [38-39]; however, their localization in the outer and inner retina [27] [30] [32-34] also indicates a role in visual processing and function.

Anesthetic potentiation of inhibitory synaptic receptors (mainly GABA_A) best matches the pharmacological profile of a wide variety of agents for producing general anesthesia in mammals [16]. All general anesthetics interact with multiple molecular targets, and characteristically, each drug has a unique pattern [18]. But though functional effects (anesthesia) of anesthetics may be equivalent, the processes at the receptor level may not be [40-50]. The degree of anesthetic potentiation and the diverse actions of general anesthetics have been shown to depend upon the subunit compositions of the GABA_A receptor subtype [16] [18]. Olsen and Sieghart [36] have alluded to more than 800 distinct GABA_A receptor subtypes that may exist within the brain introducing a multiplicity of possibilities for binding processes and optimization for potentiation and direct activation by general anesthetics, which may underlie the variable influences of pentobarbital sodium and propofol on retinal function. In fact, specific binding sites on the heteropentameric GABA_A

receptor differ for propofol and pentobarbital sodium for their respective potentiating and directly activating effects [51] and may likely contribute to their differential functional effects as we have shown in the K1 and K2.1 responses. Additionally, pentobarbitone/pentobarbital also exhibits a more widespread effect than does propofol at clinically relevant concentration levels on neuromodulatory receptors of the central nervous system [29] [51]. For example, pentobarbital modulates activity not only on GABA_A receptors [52], but also effectively inhibits neuronal nicotinic acetylcholine, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, and glycine receptors [29], all of which are found in retinal neurons [28] [31] [53-54], and when inhibited would lead to response decrements. When full-field ERGs were recorded in children under disopropofol (or propofol), b-wave amplitude and cone b-wave implicit time were nearly indistinguishable from responses recorded with topical anesthesia [55]. These findings indicate that propofol anesthesia does not result in hyperabnormal responses of (particularly outer) retinal function, but rather suggests further that pentobarbital sodium anesthesia may result in decrement in responsiveness. Although specific attribution of retinal cellular activity subserving the K2.1 waveform components has not as yet been definitively established, the lower K2.1 amplitudes (of p1 and n2) observed in this study may be accounted for by propofol effects in slowing desensitization and deactivation of the GABA_A receptors [56], which would stabilize and enhance receptor channel opening, and therefore prolong the postsynaptic inhibitory effects.

In summary, K1 and K2.1 mfERG responses of rhesus macaques are robust and reproducible when collected under either pentobarbital sodium or propofol anesthesia. K1 response density amplitude is generally larger under propofol than pentobarbital sodium anesthesia. Late-wave components (N2 and P2) are more prolonged under pentobarbital sodium than under propofol anesthesia. K1 LFC RMS SNR (larger RMS SNR values for propofol than for pentobarbital) differences were also noted to be significant in response comparisons between pentobarbital sodium and propofol. These response differences likely arise from variable relative effects of the anesthetics on retinal γ -aminobutyric acid (GABA_A), and perhaps in part on glycine and on glutamate receptors. Therefore, as this study demonstrates, one must be mindful of the differential effects on the mfERG in the usage of these anesthetics. Care must be taken in combining data collected under propofol and pentobarbital sodium anesthesia.

There are practical considerations in the use of propofol rather than pentobarbital sodium anesthesia for multifocal electrophysiological testing—propofol permits a continuous intravenous infusion versus periodic injections of pentobarbital sodium, and thus, provides a more consistent level of anesthesia during recordings. A briefer period for initial responsiveness or waking and full recovery is typically experienced [57-59]. Propofol intravenous infiltration does not result in negative ancillary tissue effects or extravasation injury [57] [58] [59] [60], which results from inadvertent infiltration of the surrounding tissue layer with injections, and may elicit a local, limited inflammatory response or an extensive necrosis of the epidermis and underlying soft tissues [61-62]. Emesis potential is much reduced with propofol when compared to pentobarbital sodium anesthesia [58-59]. Therefore, propofol is an appealing alternative anesthetic in the collection of mfERG responses in rhesus macaques.

Acknowledgments

This research was supported by National Institutes of Health grants EY014041, EY016665, and grants from the American Health Assistance Foundation, Retina Research Foundation, and Research to Prevent Blindness. Elizabeth Hennes-Beean, Allen Irgens, and Cassandra Miller provided technical assistance during the electrophysiologic procedures and monitored the animals' health throughout the study period.

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Fig. 1. Right eye (OD) filtered ring-average (arithmetic mean) waveforms for rings 1-6 (ring 1: foveal element, ring 6: $\sim 30^\circ$ retinal eccentricity) of the K1 mfERG responses with pentobarbital (PB) and propofol (PF) anesthesia. The primary wave components (N1, P1, N2, and P2) are denoted in the upper left panel. Error bars represent ± 1 SEM. Inset depicts the grouped arrangement of the hexagonal elements that encompassed the individual averaged rings

Fig. 2. OD filtered ring-average for rings 1-6 of the K2.1 mfERG responses with PB and PF anesthesia. The primary wave components (p1, n2, and p2) are denoted in the upper left panel. Conventions are the same as for Fig. 1

Fig. 3. Response density amplitude (nV/deg^2) as a function of time (ms) of the mean K1 HFC responses of rings 1-6 (central inset) representing approximately the central 30° retinal eccentricity for pentobarbital (upper) and for propofol (lower) anesthesia. Signal (10-180 ms) and noise segments (700-800 ms) are demarcated on each response plot.

Fig. 4. Root mean square signal-to-noise ratio (RMS SNR) as a function of retinal eccentricity (ring) with PF and PB anesthesia for right eye (OD) and left eye (OS). Error bars represent ± 1 SEM. Filled circles: PF OD; filled squares: PB OD; open circles: PF OS; open squares: PB OS. (a) K1 LFC (b) K1 HFC (c) K2.1 LFC

Fig. 5. Mean filtered mfERG waveforms of the OD retinal quadrants (ST, SN, IN, and IT) for the K1 responses. Error bars represent ± 1 SEM. Inset illustrates the grouped arrangement of the hexagonal elements used for evaluating the quadrant data

Fig. 6. Mean filtered mfERG waveforms of the OD retinal quadrants (ST, SN, IN, and IT) for the K2.1 responses. Conventions are the same as for Fig. 5

Fig. 7.
RMS SNR as a function of retinal quadrant with PF and PB anesthesia for OD and OS.
Conventions are the same as for Fig. 4. (a) K1 LFC (b) K1 HFC (c) K2.1 LFC