

Effects of common anesthetics on eye movement and electroretinogram

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Abstract High-resolution magnetic resonance imaging (MRI) provides non-invasive images of *retinal* anatomy, physiology, and function with depth-resolved laminar resolution. Eye movement and drift, however, could limit high spatial resolution imaging, and anesthetics that minimize eye movement could significantly attenuate retinal function. The aim of this study was to determine the optimal anesthetic preparations to minimize eye movement

and maximize visual-evoked retinal response in rats. Eye movements were examined by imaging of the cornea with a charge-coupled device (CCD) camera under isoflurane, urethane, ketamine/xylazine, and propofol anesthesia at typical dosages in rats. Combination of the paralytic pancuronium bromide with isoflurane or ketamine/xylazine anesthesia was also examined for the eye movement studies. Visual-evoked retinal responses were evaluated using full-field electroretinography (ERG) under isoflurane, ketamine/xylazine, urethane, and ketamine/xylazine + pancuronium anesthesia in rats. The degree of eye movement, measured as displacement per unit time,

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was the smallest under 1% isoflurane + pancuronium anesthesia. The ketamine/xylazine groups showed larger dark-adapted ERG a- and b-waves than other anesthetics tested. The isoflurane group showed the shortest b-wave implicit times. Photopic ERGs in the ketamine/xylazine groups showed the largest b-waves with the isoflurane group showing slightly shorter implicit times at the higher flash intensities. Oscillatory potentials revealed an early peak in the isoflurane group compared with ketamine/xylazine and urethane groups. Pancuronium did not affect the a- and b-wave, but did increase oscillatory potential amplitudes. Compared with the other anesthetics tested here, ketamine/xylazine + pancuronium was the best combination to minimize eye movement and maximize retinal function. These findings should set the stage for further development and application of high-resolution functional imaging techniques, such as MRI, to study retinal anatomy, physiology, and function in anesthetized rats.

Keywords Anesthesia · Eye movement · ERG · fMRI · Magnetic resonance imaging

Introduction

In vivo investigations of the retinas in animal models are routinely performed under general anesthesia for easy restraint and to control anxiety. Stable and adequate anesthesia is important because data acquisition may not be instantaneous, data may need to be acquired in time series to detect temporal responses to multiple stimuli, and/or multiple types of clinically relevant data may need to be acquired in a single setting. However, many of the anesthetic agents used in retinal studies alter retinal function [1–7] and do not completely remove eye movements [8–10]. A systematic evaluation of commonly used anesthetic agents may be helpful to facilitate experimental investigation of retinal function in vivo.

Eye movements are generally classified as gaze-stabilizing or gaze-shifting mechanisms. Gaze-stabilizing mechanisms, such as the vestibule ocular reflex and optokinetic reflex, keep the object of interest in the field of vision when the head or the object is

moving. Gaze-shifting mechanisms involve involuntary eye movements such as saccades, pursuit, and vergence [11]. Under light general anesthesia, the eye moves in slow oscillatory sweeps due to the incomplete recovery from saccades. For example, optokinetic studies in rabbits and monkeys [12, 13] showed that eye movements under ketamine were coordinated to the visual input with the activation of the sensorimotor loop. Increased depth of anesthesia can generally reduce eye movements, although not always effective. For example, microsaccadic motion has been observed even under deep anesthesia [14, 15]. Moreover, different anesthetics may have different effects on eye movements [9, 16].

Dosages and the types of anesthetics are also important considerations for minimizing their effects on retinal functions. High dose of anesthetics could markedly attenuate retinal function, and thus, evoked functional responses [1–7]. A number of studies have noted various effects of different anesthetic types and doses on retinal function measured with electroretinograms (ERG) [5, 7, 17–20]. With increasing interest in functional imaging of the retina using emerging techniques such as optical imaging [21–24], functional MRI [25–30], blood flow MRI [31–34], and optical coherence tomography [35–37] in animal models, the ability to maintain stable eye position and preserve optimal retinal function is important.

The goals of this study were to evaluate several commonly used anesthetics at their typical dosages for their effectiveness in suppressing eye motion and their effects on retinal functions. The anesthetics studied include isoflurane, urethane, ketamine/xylazine, propofol, isoflurane + pancuronium bromide (a paralytic), and ketamine/xylazine + pancuronium bromide. Eye movements were evaluated using optical recording of the corneal surface via a CCD camera. Retinal function was evaluated using scotopic and photopic ERGs. While the primary purpose for this study was to determine the optimal conditions for achieving high-resolution magnetic resonance imaging (MRI) that provides non-invasive images of *retinal* anatomy, physiology, and function with depth-resolved laminar resolution [25–34], these findings may be applicable to many other experimental investigations of the retina.

Methods

All experiments were performed with the approval of Institutional Animal Care and Use Committees (IACUC) at Emory University and the Atlanta Veterans Affairs Medical Center, and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Two sets of experiments were performed on separate groups of male Sprague–Dawley rats: one to evaluate eye movement studies using a CCD camera and the other to evaluate retinal function responses using ERG with Ganzfeld stimuli.

Eye movement measurements

Animal preparation

Six groups of male Sprague–Dawley rats (3 months old, 275–350 g) were investigated: (1) 1% isoflurane ($n = 6$, recommended standard dose for isoflurane is 1–3%), (2) 2% isoflurane ($n = 6$), (3) urethane (1 g/kg body weight, i.p., $n = 6$, AD50 of 1 g/kg), (4) ketamine/xylazine (75 mg/kg and 10 mg/kg, respectively, i.p., every 30 min, $n = 6$, AD50 between 47.5 mg/kg to 123 mg/kg of ketamine i.p.), (5) propofol (45 mg/kg/hr continuous i.v. infusion, $n = 6$, AD50 of 30 mg/kg/h), (6) 1% isoflurane + pancuronium bromide (3 mg/kg first dose, 1 mg/kg/hr, i.p., $n = 4$), and (7) ketamine/xylazine + pancuronium bromide ($n = 5$). Rats were anesthetized, orally intubated with a 14-gauge catheter, mechanically ventilated, and immobilized in a stereotaxic head frame. End-tidal CO_2 was monitored throughout the experiment and maintained within normal physiological ranges. Rectal temperature was monitored and maintained between 37.5 and 38.5°C using feedback circulating warm water pad. Blood pressure was monitored continuously via a catheter in the femoral artery connected to a pressure transducer (Harvard Instruments, Holliston, MA). In the propofol group, the femoral vein was catheterized for the administration of the anesthetic. In the isoflurane group, the arterial catheterization was performed under 2% isoflurane for blood pressure monitoring and the anesthetic level was turned down to the desired level after surgery. Rats were transferred to a stereotaxic device to immobilize the head and allowed to stabilize for 30 min after setup before proceeding to

eye movement recordings. Finally, a small mark was made on the cornea using a black permanent marker pen for quantitative tracking of eye movements.

CCD recording

Eye movements were monitored using an optical imaging instrument (Imager 3001, Optical Imaging Inc., Rehovot, Israel) which was equipped with a CCD camera (7.4 $\mu\text{m}/\text{pixel}$ resolution). Recordings were repeated 4 times with a gap of 10 min between recordings. Magnification of up to 4 \times could be achieved with this arrangement. The final resolution of the image was determined for every setup by taking a photograph of a millimeter scale. To minimize the movement of the eye relative to the camera, the camera was mounted on the same platform as the stereotaxic headset. Movies were recorded at two different frame rates: 1 frame/s for 4 min (low frame rate, LFR) and 25 frames/s for 20 s (high frame rate, HFR). LFR recordings were performed in all groups. HFR recordings were done on rats in the isoflurane + pancuronium group at different respiration rates as modulated by the mechanical ventilator.

Data analysis

Recordings of eye movements were analyzed using programs written in Matlab (Mathworks, Natick, MA). For the LFR data, the frame-by-frame displacement of the center-of-mass of the black ink spot was calculated in units of mm/s as a measure of eye movement (motion index), and the eye movement was plotted on a graph (movement pattern). The distances between the center-of-mass coordinates in each frame were also plotted as a time series (movement waveform). For the HFR data, Fourier transform was applied to the movement waveform data to determine its frequency components. Two-tailed *t*-tests were used for statistical testing, and a $P < 0.05$ was considered to be statistically significant.

Retinal function measurements

Animal preparation

Four groups of rats (male Sprague–Dawley, 3 months age, 275–300 g) were investigated using the same

anesthetic dosages as described above: (1) 1% isoflurane ($n = 4$), (2) urethane ($n = 4$), (3) ketamine/xylazine ($n = 4$), (4) ketamine/xylazine + pancuronium bromide ($n = 6$). Rats were dark adapted overnight. Oral intubation was performed under dim red lighting in the ketamine/xylazine + pancuronium group after the induction of ketamine/xylazine anesthesia. The rats were setup on a ventilator and end-tidal CO₂ monitored, followed by i.p. administration of pancuronium. For all groups, physiological parameters were monitored and maintained within normal physiological ranges as described above. Eye drops were applied and they included 0.5% proparacaine HCl for topical anesthetic, and 2.5% phenylephrine HCl, 1% tropicamide (Mydracyl), and 1% cyclopentolate (Alcon, Fort Worth, Texas) for pupil dilation. A silver-impregnated nylon fiber, placed on the surface of the cornea after application of methylcellulose (for lubricating and hydrating the cornea), was used as the recording electrode [38, 39]. Ground and reference subdermal needle electrodes were inserted into the tail and both cheeks, respectively. The impedance of the electrodes was checked periodically and maintained around 20 k Ω by repositioning electrodes or with application of methylcellulose.

ERG recordings

Five steps of scotopic stimuli were presented to the rat using a Ganzfeld (UTAS E-3000, LKC systems, Gaithersburg, MD), starting with the lowest time integrated luminance of 0.00095 cd s/m², followed by 0.0155, 0.249, 4.1, and 137 cd s/m². Luminance was calibrated using a radiometer (IL1700 Radiometer, International Light, Newburyport, MA) with the scotopic and photopic filter for dark- and light-adapted flashes, respectively. Interstimulus intervals ranged from 4.1 s at the lowest stimulus strength to 65.1 s at the brightest stimulus. The scotopic equivalent for the strongest photopic calibrated stimulus (81 cd s/m²) was 54 cd s/m². A gap of 1–2 min was given between stimulation steps. Following 10 min of light adaptation with a rod-saturating background (30 cd/m²), photopic stimuli were presented at 0.476 Hz, with 0.151, 0.961, 1.9, and 75 cd s/m² stimuli. ERG waveforms from both eyes were band-pass filtered from 1 to 1,500 Hz (standard protocol) and sampled onto a computer for further analysis (UTAS E-3000, LKC systems, Gaithersburg, MD).

Data analysis

ERG waveforms were analyzed by measuring a-wave and b-wave amplitudes and implicit times. The a-wave was measured from baseline to the trough of the first-negative peak and the b-wave was measured from the trough of the a-wave to the peak of the large positive wave, excluding the OP peaks. OPs were extracted from the scotopic ERG recorded in response to the 4.1 cd s/m² stimulus using a band-pass filter of 75–500 Hz. This time integrated luminance was chosen because the OP component wavelets were more distinctly visible and a-wave interference was minimal. The OP wavelets were numbered OP1 through OP3, starting at the first detected positive peak. Individual OP amplitudes (peak-positive amplitude and peak-negative amplitude of previous peak) and latencies were determined from both eyes in each rat. No differences were found between left and right eyes using paired *t* tests and thus, values for only left eyes are presented. Statistical analysis between treatment groups was performed using repeated measures ANOVA or one-way ANOVA (SigmaStat 3.5, Chicago, IL) with $P < 0.05$ taken to be statistically significantly different.

Results

Animal physiology

Heart rate and mean arterial blood pressure (MABP) recorded from all groups in the eye movement studies were within the normal physiological ranges. Following pancuronium administration, heart rate increased from 380 ± 30 to 428 ± 5 bpm ($P < 0.01$) and MABP dropped from 120 ± 9 to 108 ± 5 mmHg ($P < 0.01$).

Eye movement measurements

Low frame-rate recording

The iris and the black spot on the cornea were clearly visible on images obtained using the CCD camera, facilitating quantification of frame-to-frame eye motion. Representative patterns of motion recorded over 4 min under different anesthetics are shown in Fig. 1. The eye was seen to move in a slow rolling

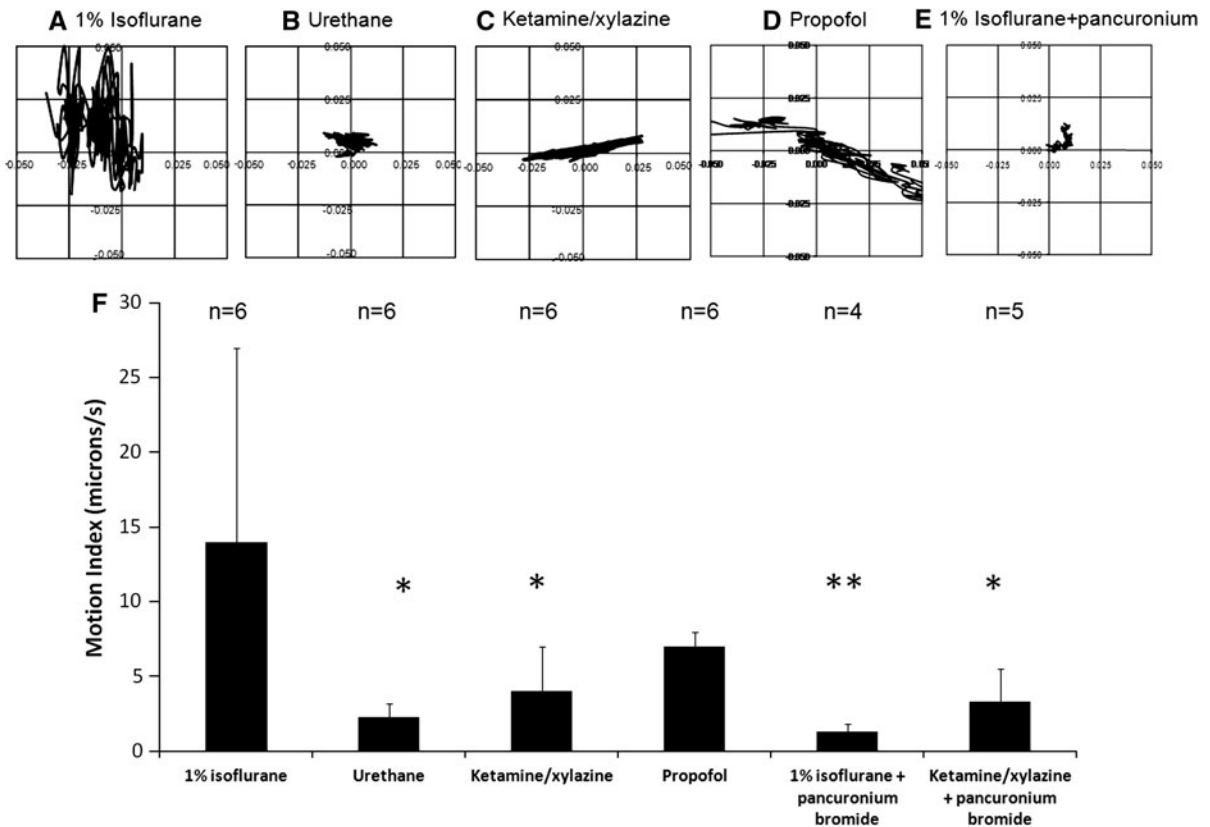


Fig. 1 Representative traces of eye movement in Sprague–Dawley rats anesthetized with (A) isoflurane (1% in air, inhalation), (B) urethane (1,000 mg/kg, i.p.), (C) ketamine/xylazine cocktail (75 and 10 mg/kg, respectively, i.p.), (D) propofol (45 mg/kg/hr, i.v. infusion), and (E) 1% isoflurane + pancuronium (3 mg/kg, i.p.). These traces were generated from movies recorded over 4-minute duration, at 1 frame-per-second (low frame rate). (F) Group averaged motion

index (average distance moved by the eye in 4 min, in mm/s \pm SD) in rats in various anesthetic groups. X- and y-axes are in mm for (A) through (E), $N = 6$ all groups except $N = 4$ for 1% isoflurane with pancuronium and $N = 5$ for ketamine/xylazine with pancuronium. * $P < 0.05$ and ** $P < 0.01$ compared to 1% isoflurane anesthesia). Panels (A) and (E) previously appeared in a review paper and is reprinted with permission from Jpn J Ophthalmol. 2009; 53: 352-367 [64]

fashion under isoflurane anesthesia, similar to Stage 1 or light sleep [15]. Preliminary data showed the amplitude of these oscillatory eye movements became larger and with reduced frequency as the isoflurane level increased from 1 to 2%. Thus, 2% isoflurane group was not further analyzed. In the urethane, ketamine/xylazine, 1% isoflurane + pancuronium, and ketamine/xylazine + pancuronium groups, the amplitudes of the eye movements were comparatively smaller but with higher frequencies. All movements were confirmed to originate from the movement of the eye within the orbit by the relative movement of the black ink spot with respect to extra-orbital landmarks, such as the supra-orbital ridge.

Among the anesthetics tested, motion index was the highest under 1% isoflurane and propofol with

motion indices of 14 ± 13 and 7 ± 1 microns/s, respectively (Fig. 1F). The eye movement was significantly smaller under urethane, ketamine/xylazine, and ketamine/xylazine + pancuronium bromide with motion indices of 2.3 ± 0.9 , 4 ± 3 , and 3 ± 2 microns/s, respectively ($P < 0.05$). Rats anesthetized with 1% isoflurane + pancuronium showed smallest eye movement among all anesthetics tested, with motion index under 1.3 ± 0.5 microns/s ($P < 0.01$).

High frame-rate recording:

Fourier decomposition was performed on time-course data from rats anesthetized with 1% isoflurane + pancuronium to correlate with possible sources of the

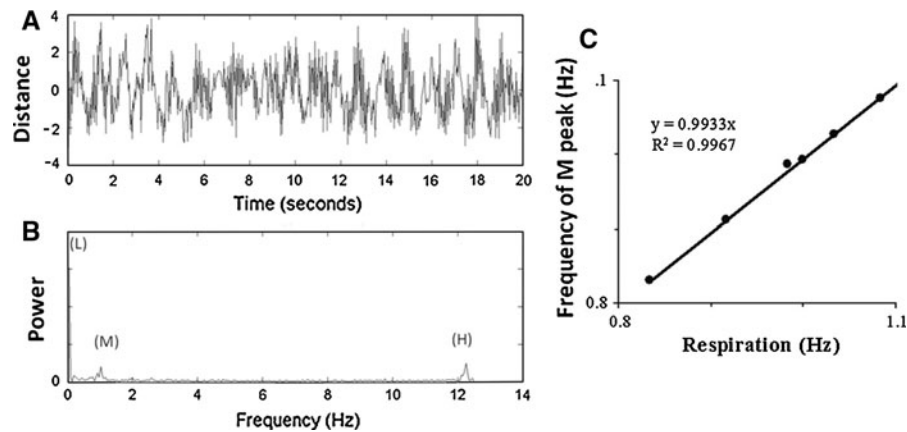


Fig. 2 (A) Representative eye movement waveforms (position of the eye with respect to the initial frame) and (B) frequency components in the movement waveform in (A) determined by Fourier transformation in a high frame-rate recording (25 frames-per-second). Two peaks were consistently detected in

the frequency decomposition at ~ 1 Hz (M), and 12.2 Hz (H). (C) The mid-frequency component (M) was highly correlated with respiration rate ($R^2 = 0.99$, $P < 0.01$). Data were obtained from rats anesthetized with 1% isoflurane + pancuronium (3 mg/kg, i.p.)

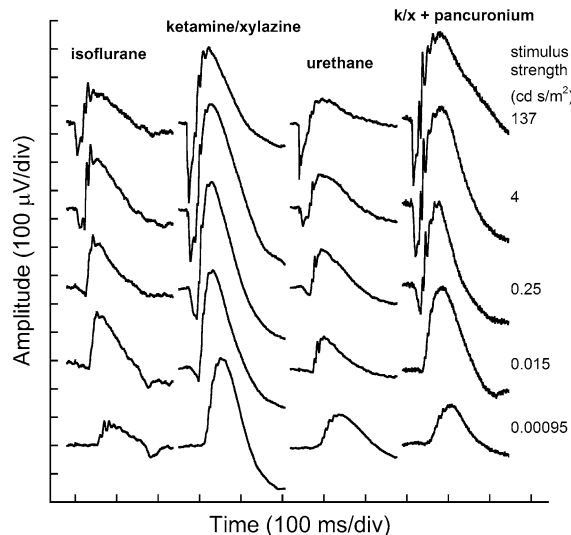


Fig. 3 Stacked plots of representative scotopic ERG waveforms recorded from rats anesthetized under isoflurane (1% in air, inhalation), ketamine/xylazine cocktail (75 and 10 mg/kg, respectively, i.p.), urethane (1,000 mg/kg, i.p.), or ketamine/xylazine + pancuronium (K/X + pancuronium; ketamine: 75 and 10 mg/kg, respectively, i.p.; pancuronium: 3 mg/kg, i.p.). The stimulus strengths are indicated at the right side of the graph

movement (Fig. 2). The Fourier spectrum showed two distinct peaks: ~ 1 Hz (mid-frequency component) and ~ 12.2 Hz (high-frequency component), in addition to the zero frequency peak. The mid-frequency component was linearly correlated with respiratory frequency with an R^2 of 0.99 ($P < 0.01$). The high-frequency component did not vary with

respiratory nor cardiac pulse rates (the cardiac peak would have shown up at ~ 7 Hz).

Retinal function measurements

Scotopic stimulation

Representative scotopic ERG waveforms from each anesthetic group are shown in Fig. 3. While a-, b- and OP waves could be clearly identified in each anesthetic group, the isoflurane and ketamine/xylazine + pancuronium groups showed more prominent OP waves. The a-wave amplitude increased with increasing stimulus strength as expected. The b-wave amplitude, on the other hand, increased moderately across the brighter time integrated luminances.

The scotopic a-wave amplitudes from the ketamine/xylazine groups were consistently greater than those from the isoflurane and urethane groups across all time integrated luminances (Repeated ANOVA $F(12, 89) = 5.26$, $P < 0.001$; Fig. 4A; Table 1). The latency of a-wave of the ketamine/xylazine groups was significantly shorter from other anesthetic groups (Repeated ANOVA $F(9, 70) = 4.40$, $P < 0.001$, Fig. 4B) at the lowest flash stimulus strength (0.0155 cd s/m²). In response to the brightest stimulus, the latency was similar for all three groups (Table 1).

The scotopic b-wave amplitudes from the ketamine/xylazine groups were significantly higher than the other groups at the brightest time integrated

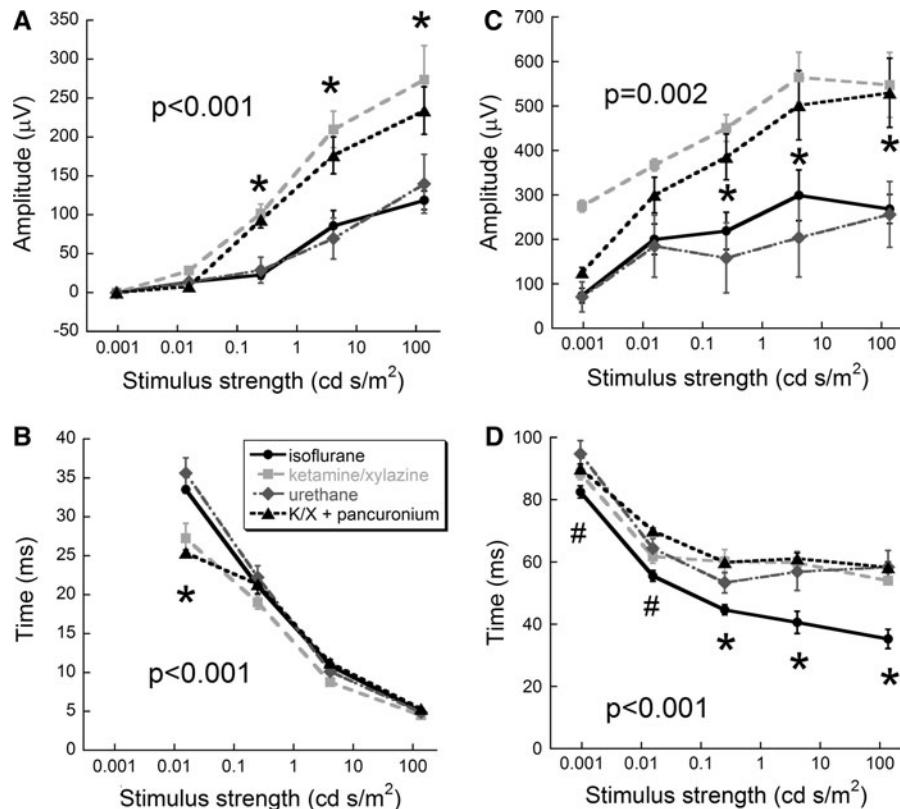


Fig. 4 Average dark-adapted a- and b- wave amplitude and latency in rats anesthetized with isoflurane (1% in air, inhalation; $n = 4$), ketamine/xylazine (75 and 10 mg/kg, respectively, i.p., $n = 4$), urethane (1,000 mg/kg, i.p., $n = 4$), or ketamine/xylazine + pancuronium (K/X + pancuronium; ketamine:75 and 10 mg/kg, respectively, i.p.; pancuronium: 3 mg/kg, i.p., $n = 6$). Ketamine/xylazine anesthetized rats had greater (A) a-wave amplitudes and (B) shorter a-wave latency in most scotopic stimulations. Similarly, (C) b-wave amplitude was significantly greater in ketamine/xylazine groups compared

with isoflurane or urethane groups across all time integrated luminances. However, the (D) b-wave latency was shorter in the isoflurane group compared with other groups in response to the brighter stimulus strengths. P values were obtained from repeated ANOVAs with asterisks indicating significant post hoc comparisons. In panel D, the isoflurane group is significantly shorter than urethane (step 1) or K/X + pancuronium (step 2) groups as indicated by # and significantly shorter than all ketamine/xylazine and urethane groups where indicated by *

luminances (Repeated ANOVA $F(12, 89) = 3.04$, $P = 0.002$, Fig. 4C; Table 1). Unlike a-wave latency, the b-wave latency was considerably shorter in the isoflurane anesthetized rats for the three brightest flash stimuli compared with the other groups (0.0155–137 cd s/m²; Repeated ANOVA $F(12, 89) = 5.12$, $P < 0.001$, Fig. 4D; Table 1). The average b-wave latency was not significantly different between the ketamine/xylazine and urethane groups.

Photopic stimulation

Representative photopic ERG waveforms from each anesthetic group are shown in Fig. 5. The b-wave and

OPs can be clearly identified in each group, and the amplitudes of these waves increased with time integrated luminance, as expected. Similar to the scotopic recordings, the average b-wave amplitudes were the largest in the ketamine/xylazine groups (Repeated ANOVA $F(9, 71) = 4.74$, $P < 0.001$; Fig. 6A; Table 1). The b-wave amplitudes were not statistically different between the isoflurane and urethane groups. The b-wave latency did not show any significant interaction effects, but the isoflurane group was significantly shorter than the ketamine/xylazine + pancuronium group (Repeated ANOVA, main effect, $F(3, 70) = 5.77$, $P = 0.0009$; Fig. 6B; Table 1).

Table 1 Summary of ERG wave amplitudes and latencies from 1% isoflurane, ketamine/xylazine, urethane, and ketamine/xylazine + pancuronium (K/X + pancuronium) groups

Anesthesia group	Scotopic a-wave	Scotopic b-wave	Photopic b-wave	OP1	OP2	OP3
Amplitude (μV)						
Isoflurane	119 \pm 24	268 \pm 65	68 \pm 23	172 \pm 66 [#]	137 \pm 7	30 \pm 12
Ketamine/Xylazine	274 \pm 89*	547 \pm 146 [#]	152 \pm 57*	68 \pm 27	147 \pm 8	60 \pm 19 [#]
Urethane	140 \pm 76	256 \pm 148	61 \pm 23	41 \pm 28	49 \pm 28 [#]	14 \pm 3
K/X + pancuronium	233 \pm 75	502 \pm 191	136 \pm 52	149 \pm 67	280 \pm 94	86 \pm 29
Latency (ms)						
Isoflurane	5.0 \pm 0.4	35 \pm 6*	42 \pm 6	26.7 \pm 1	35 \pm 2	48 \pm 4
Ketamine/Xylazine	4.5 \pm 0.4 [#]	54 \pm 3	50 \pm 5	25 \pm 1	31 \pm 2	44 \pm 3
Urethane	4.9 \pm 0.3	58 \pm 11	56 \pm 16	28 \pm 5	36 \pm 6	48 \pm 5
K/X + pancuronium	5.3 \pm 0.3	58 \pm 4.3	56 \pm 4	19 \pm 1	27 \pm 1	39 \pm 1

Values are mean \pm SD. Scotopic a- and b-wave values were obtained from responses to 137 cd s/m² flash. Photopic b-wave values were obtained from responses to 75 cd s/m² flash. OP values were obtained from responses to a scotopic 4.1 cd s/m² flash. $N = 4$ rats per group, except K/X + pancuronium where $N = 6$. (Repeated ANOVA, * $P < 0.001$; # $P =$ or < 0.01 ; @ $P < 0.05$)

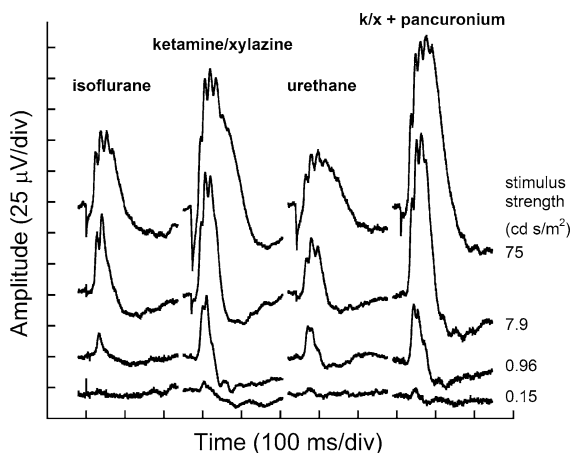


Fig. 5 Representative photopic ERG waveforms recorded from rats anesthetized with isoflurane (1% in air, inhalation), ketamine/xylazine cocktail (75 and 10 mg/kg, respectively, i.p.), urethane (1,000 mg/kg, i.p.), or ketamine/xylazine + pancuronium (K/X + pancuronium; ketamine: 75 and 10 mg/kg respectively, i.p.; pancuronium: 3 mg/kg, i.p.). Time integrated luminances are indicated on the right side of the graph

OP analysis from scotopic 4.1 cd s/m² stimulation

OP-waveforms were extracted from the ERG waves obtained for scotopic 4.1 cd s/m² stimulation using band-pass filters. Representative OP-waveforms from different anesthesia groups are shown in Fig. 7A. There were visible differences in the OP-waveforms (larger OPs) of the isoflurane and ketamine/xylazine + pancuronium groups compared with the other

groups. OP wavelets were numbered from OP1 through OP3 starting from the first detected positive peak (Fig. 7A, isoflurane waveform). Note that the largest OP wavelet in the isoflurane group was OP1, while the largest OP wavelet in the ketamine/xylazine and urethane groups was OP2.

The amplitude of OP1 was significantly higher in isoflurane group compared with either the urethane or ketamine/xylazine (only) groups (one-way ANOVA $F(3, 17) = 7.24$, $P = 0.004$; Fig. 7B; Table 1). The amplitude of OP2 was largest for the ketamine/xylazine + pancuronium group while those of the ketamine/xylazine and isoflurane group were similar (one-way ANOVA $F(3, 17) = 10.93$, $P < 0.001$; Fig. 7B; Table 1). OP3 from each group showed progressively reduced amplitudes. The ketamine/xylazine + pancuronium group had significantly larger OP3 waves than the isoflurane and urethane groups (one-way ANOVA $F(3, 17) = 7.63$, $P = 0.003$; Fig. 7B; Table 1).

A trend for shorter implicit times in the ketamine/xylazine group across all OPs compared with other anesthetic groups was observed, with pancuronium producing even shorter responses. Ketamine/xylazine + pancuronium was shorter than urethane and isoflurane for OP1 (Kruskal–Wallis one-way ANOVA on Ranks, $P < 0.05$) than urethane for OP2 (one-way ANOVA $F(3, 17) = 5.08$, $P = 0.01$) as well as urethane and isoflurane for OP3 (one-way ANOVA $F(3, 17) = 9.76$, $P < 0.001$; Table 1).

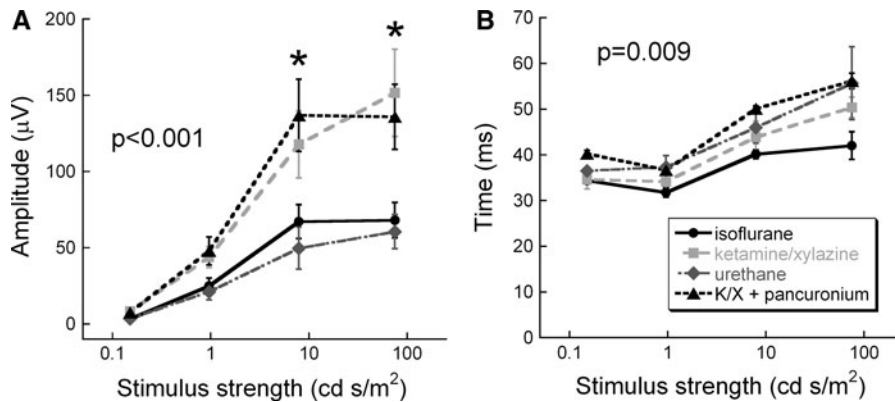


Fig. 6 Average light-adapted b-wave. amplitude (A) and latency (B) in rats anesthetized with isoflurane (1% in air, inhalation, $n = 4$), ketamine/xylazine (75 and 10 mg/kg, respectively, i.p., $n = 4$), urethane (1,000 mg/kg, i.p., $n = 4$), or ketamine/xylazine + pancuronium (K/X + pancuronium; ketamine: 75 and 10 mg/kg, respectively, i.p.; pancuronium:

3 mg/kg, i.p., $n = 6$). The b-wave amplitude was greater in the ketamine/xylazine groups (A), while the isoflurane group had significantly shorter in b-wave latency (B) than the other anesthetic groups. (P values are from repeated ANOVAs with asterisks indicating significant post hoc comparisons.)

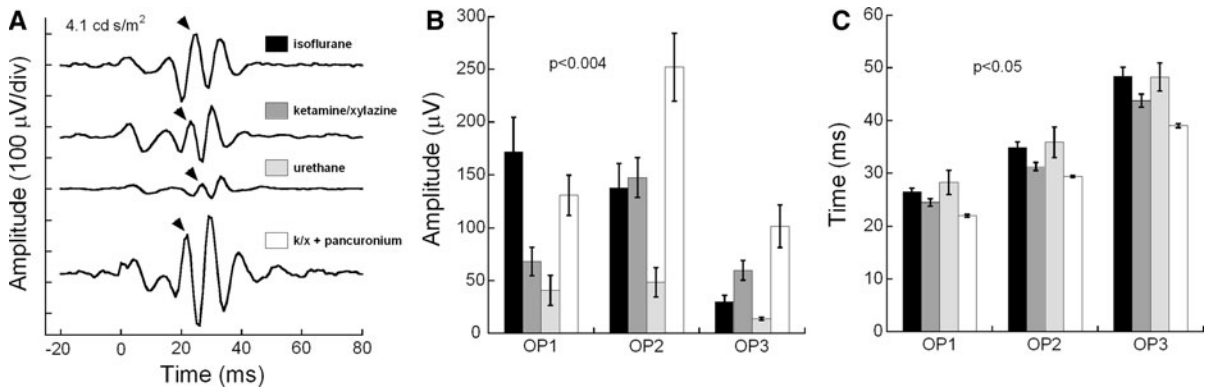


Fig. 7 (A) Representative oscillatory potentials (OP), extracted from raw ERGs elicited from a stimulus of 4.1 cd s/m² under scotopic conditions, using a band-pass filter (cut off frequencies of 75 and 500 Hz), from rats anesthetized under isoflurane (1% in air, inhalation), ketamine/xylazine (75 and 10 mg/kg, respectively, i.p.), urethane (1,000 mg/kg, i.p.), or ketamine/xylazine + pancuronium (K/X + pancuronium; ketamine:75 and 10 mg/kg, respectively, i.p.; pancuronium: 3 mg/kg, i.p.).

The arrowheads indicated OP1 in each waveform. Group-averaged (B) amplitude and (C) latency of OP wavelets (OP1 through OP3) showed greater OP wavelet amplitudes in rats anesthetized with isoflurane for OP1 and ketamine/xylazine + pancuronium for OP2, and OP3. P values indicate significant differences for all OP wavelets as calculated with one-way ANOVAs

Discussion

This study reports the effects of a few commonly used anesthetics at typical dosages on eye movement and retinal function in rats. Eye movements were the largest under isoflurane and smallest under urethane. With the addition of a paralytic, eye movements were markedly reduced. Robust ERGs were detected in all anesthetic groups. The ketamine/xylazine groups showed the largest a- and b-wave amplitudes.

The isoflurane group showed the largest OP1 amplitudes and shortened b-wave and OP latencies. Pancuronium reduced eye movements and enhanced inner retinal function, as measured by OP2 amplitude. Different anesthetics had different effects on eye movement and retinal function. Ketamine/xylazine + pancuronium was the most effective at minimizing eye movements and maximizing retinal function in these studies. Due to these different effects, the choice of anesthetic must be carefully considered to reduce

eye movements and minimize the effects on specific ERG components of retinal function. These results could have important implications for high-resolution MRI and other studies of the retina in animal models.

Effect of anesthesia on eye movement

Several studies have shown that most anesthetics decrease peak saccadic velocity in a dose-dependent manner, but this decrease in velocity is dependent on the type of anesthetic used [8, 9, 16, 40]. Peak saccadic velocity was found to be depressed in a dose-dependent fashion at concentrations of 0.6 and 1.5 mg/kg/h, 15–25 min after i.v. infusion of propofol [8], and 9.5 and 14.1 maximum alveolar concentration (MAC) of isoflurane in humans [40]. The peak saccadic velocity was found to decrease linearly with \log_{10} propofol concentrations in the blood [40]. Peak saccadic velocity was depressed to a significantly greater level by cyclopropane than halothane at similar MAC [16], while peak saccadic velocity did not change with increasing concentrations of nitrous oxide, even at anesthetic levels of 5 and 10% MAC [9].

However, eye movements under anesthesia are not limited to saccadic movements. Large amplitude oscillatory eye movements were observed in rats anesthetized with isoflurane, ketamine/xylazine, and propofol while those under urethane anesthesia could be classified mainly as saccadic (Fig 1). These observations suggest a need for a better measure of eye movement than peak saccadic velocity and latency to describe eye movement under anesthesia for multimodal MRI where multiple repeated data acquisitions could occur over relatively long duration (minutes to hours). For the analysis herein, the displacement of the eye was calculated frame-by-frame and the pattern of eye movement was plotted and compared between anesthetics to account for both the saccadic and oscillatory movement of the eye. It should also be noted that any significant eye movement due to drift would be visible in the plots of eye movement pattern.

Among the anesthetics studied here, isoflurane in the absence of paralytic yielded the largest degree of eye movement. Isoflurane is a commonly used anesthetic in electrophysiological recordings and functional MRI because a steady dose can be readily maintained throughout the studies and isoflurane is compatible with survival studies [41, 42]. Isoflurane

+ pancuronium showed the least eye movement among the conditions tested. Furthermore, pancuronium added to ketamine/xylazine cocktail also reduces eye movements. Pancuronium bromide is a non-depolarizing competitive acetylcholine antagonist, acting primarily on postsynaptic nicotinic acetylcholine receptors of the neuromuscular junction, and it has little effects on function [43]. Pancuronium can be readily reversed by neostigmine, allowing survival and longitudinal studies. While paralytics markedly reduced eye movement, there was still some residual movement. Fourier analysis of the HFR recordings showed that the majority of the movement correlated with the respiratory rate. Respiratory effects could compromise high-resolution functional MRI directly through motion or indirectly by inducing susceptibility-induced signal fluctuations [44]. These artifacts can be eliminated by using respiratory gating in MRI acquisition or through post processing of MRI data (Fig. 2). In addition, there was also a residual 12.2 Hz in the power spectrum, which could be due to ocular microtremors or equipment noise, and which warrants further investigation.

Effect of anesthesia on ERG

Eye movement could potentially induce amplitude variations in ERG due to either change in the contact impedance of the ERG fibers with the cornea or variations in incident flash intensity. It was noted that while ketamine/xylazine and urethane caused the rat eyelids to remain open, isoflurane often caused the eyelids to partially close. Furthermore, significant eye movement in the isoflurane group could result in the movement of the corneal electrodes creating large impedance and reduced ERG signals. Thus, care was exercised in the ERG setup. First, the contact impedance of the ERG electrodes was tested frequently, and maintained around 20 k Ω , by repositioning electrodes or with application of methylcellulose. Second, lid speculums were used to keep the eyes open in the isoflurane group. Third, the rat head was placed into the uniform field Ganzfield so as to illuminate the eyes with uniform light intensity, independent of the direction in which the eye is pointing. Lastly, as a control, the ERG waves from each step were normalized to the a-wave amplitude, so that any reduction in amplitude due to systemic errors such as large impedance could be eliminated. The results of such

analysis (data not shown) were not different from those obtained without normalization presented here, confirming that the differences between anesthetic groups were not influenced by changes to electrode impedances.

While the various anesthetics were tested here at their typical dosages in MRI or ERG studies, it is important to compare the depth of anesthesia achieved in each group to better understand the observations. The recommended dose of isoflurane to induce anesthesia is 1–3% in air, with minimum alveolar concentration (MAC) necessary to block movement in response to noxious stimulus between 1.2 and 1.4% in Wistar and Sprague–Dawley rats weighing between 350 and 600 g [45–47], and with loss of righting reflex seen at isoflurane levels as low as 0.65% in adult Fischer rats [48]. The preferred dose for ketamine/xylazine i.p. is 40–80 and 5–10 mg/kg, respectively [47], with AD50 (anesthetic dosage effective in 50% of the animals) ranging between 47.5 and 123 mg/kg i.p. when used alone in 250–400 g Sprague–Dawley rats with varying duration of effectiveness [49, 50]. The recommended infusion rate for propofol in rats is 30–55 mg/kg/hr i.v., with an AD50 of 30 mg/kg/hr in 180–200 g male Sprague–Dawley rats [51]. Similarly, the recommended dosage for urethane is 1,000–1,200 mg/kg i.p., with an AD50 of 1,000 mg/kg [52]. The dosages used herein for all anesthetics were within the recommended range, and slightly higher than the AD50.

Of the anesthetics tested with the ERG, ketamine/xylazine anesthesia provided the largest a- and b-wave amplitudes in scotopic ERG and the largest b-wave amplitude in photopic ERG, justifying its use as the preferred anesthetic for ERG studies in rats. As the dark-adapted a-wave originates from photoreceptor cells [53–55] and the b-wave originates from bipolar cells [56–58], these data did not indicate whether these anesthetics affect the photoreceptors in isolation or both the inner and outer retina simultaneously.

While urethane provided the most stable eye preparation among the anesthetics tested in the absence of a paralytic, retinal function was attenuated more than other anesthetics tested. Urethane is frequently used in many studies because it has minimal cardiovascular or respiratory effects at doses of 1000 mg/kg used herein. However, urethane is known to increase the levels of glucose and epinephrine in the

blood, especially with i.p. administrations, which could affect the neuronal response to stimuli [59, 60]. Urethane appeared to attenuate OP amplitudes far greater than the other anesthetics studied. Given that OPs are generated by the inner retina, urethane may selectively reduce inner retinal function that might affect experiments involving visual stimuli. Additionally, animals do not generally recover from urethane anesthesia, which makes urethane unsuitable for longitudinal studies.

Interestingly, two of the anesthetics produced a selective effect on inner retinal components of the ERG. The highest OP1 amplitude and earliest b-wave implicit time were measured in the isoflurane group. The OP waves could originate from the inhibitory feedback circuits of the inner retinal layers [61], while the b-wave originates from depolarizing bipolar cells which are also located in the inner retina. The addition of pancuronium bromide produced greater OPs compared with the ketamine/xylazine group alone (Fig. 7). Pancuronium bromide, a non-depolarizing competitive acetylcholine antagonist, acts primarily on postsynaptic nicotinic acetylcholine receptors [62], and the increase in OP amplitudes in this group could be a direct result of postsynaptic inhibition of cholinergic amacrine cells. If so, these findings may suggest that the cellular generators of OP2 are, at least in part, from cholinergic amacrine cells. Thus, these results suggest that anesthetics can have selective effects on inner retinal cell function.

Other studies in the literature also identified differences in how anesthetics affect retinal function, as well as species differences. Isoflurane was found to elicit shorter b-waves in mice and larger amplitude OPs [42] as found here. In contrast, while this study found ketamine/xylazine to elicit the maximal response, mice did not show the same effect [42], suggesting potential species differences. Furthermore, it has been shown that halothane or isoflurane anesthesia used in combination with nitrous oxide can reduce the photopic a- and b-wave amplitudes and latencies as well as the scotopic b-wave amplitude in humans, compared with a normal awake state [2, 5]. Similar to the findings here, the b-wave amplitudes and a- and b-wave latencies were reported to decrease under isoflurane anesthesia in dogs compared with tiletamine/zolazepam [4]. Finally, some anesthetics have been shown to have a dose-dependent manner on retinal function such as methoxyflurane, halothane, and enflurane in

rabbits [3]. A recent study showed time-dependent increase in scotopic a- and b-wave amplitudes after ketamine/xylazine anesthesia in the presence of both atropine and phenylephrine [63]. While dynamic variations in ERG amplitudes were not studied herein, it is possible that the individual ERG waveforms were not maximal. However, every effort was made to ensure uniformity in ERG acquisition across animals in the group, and only after complete induction (approximately 15 min) of anesthesia.

Conclusion

This study reports the effects of a few commonly used anesthetics at their typical dosages on eye movements and retinal function in rats. Different types of anesthetics have different effects on eye movement and ERG. Although paralytics were not tested with all anesthetics, a paralytic is, in principle, ideal for minimizing eye movement and allowing optimal functional responses from rat retinas under anesthesia. However, our results indicate some caution is warranted with the use of pancuronium bromide as it may alter inner retinal function. In these studies, the combination of ketamine/xylazine + pancuronium was most effective in minimizing eye movements and maximizing retinal function. Future studies could include other common anesthetics and dose-dependent effects. These findings are expected to set the stage for further development of depth-resolved anatomical, physiological, and functional MRI of the retina [64–66] as well as other functional studies in animal models where anesthetic is needed for immobilization.

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References

- Granit R (1933) The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. *J Physiol* 77(3):207–239
- Raitta C, Karhunen U, Seppäläinen AM, Naukkarinen M (1979) Changes in the electroretinogram and visual evoked potentials during general anaesthesia. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 211(2):139–144
- Tashiro C, Muranishi R, Gomyo I, Mashimo T, Tomi K, Yoshiya I (1986) Electroretinogram as a possible monitor of anesthetic depth. *Graefes Arch Clin Exp Ophthalmol* 224(5):473–476
- Lin SL, Shiu WC, Liu PC, Cheng FP, Lin YC, Wang WS (2009) The effects of different anesthetic agents on short electroretinography protocol in dogs. *J Vet Med Sci* 71(6):763–768
- Tremblay F, Parkinson JE (2003) Alteration of electroretinographic recordings when performed under sedation or halogenate anesthesia in a pediatric population. *Doc Ophthalmol* 107(3):271–279
- Nair G, Duong TQ (2004) Echo-planar BOLD fMRI of mice on a narrow-bore 9.4 T magnet. *Magn Reson Med* 52(2):430–434
- Sandalon S, Ofri R (2009) The effect of topical anesthesia on the rat electroretinogram. *Doc Ophthalmol* 118(2): 101–108
- Gao F, Mapleson WW, Vickers MD (1991) Effect of sub-anaesthetic infusions of propofol on peak velocity of saccadic eye movements. *Eur J Anaesthesiol* 8(4):267–276
- Gao F, Marshall RW, Vickers MD (1991) Effect of low concentrations of nitrous oxide and isoflurane on peak velocity of saccadic eye movements. *Br J Anaesth* 66(2): 179–184
- Aschoff JC (1968) The effect of diazepam (Valium) on the saccadic eye movements in man. *Arch Psychiatr Nervenkr* 211(4):325–332
- Meier RK, Dieringer N (1993) The role of compensatory eye and head movements in the rat for image stabilization and gaze orientation. *Exp Brain Res* 96(1):54–64
- Dellepiane M, Mora R, Salami A (2007) Vestibular and optokinetic nystagmus in ketamine-anesthetized rabbits. *Int Tinnitus J* 13(1):15–20
- Leopold DA, Plettenberg HK, Logothetis NK (2002) Visual processing in the ketamine-anesthetized monkey. Optokinetic and blood oxygenation level-dependent responses. *Exp Brain Res* 143(3):359–372
- Laborit G, Angiboust R, Papin JP (1977) A study of eye movement for assessing recovery from anaesthesia. *Br J Anaesth* 49(8):805–810
- Power C, Crowe C, Higgins P, Moriarty DC (1998) Anaesthetic depth at induction. An evaluation using clinical eye signs and EEG polysomnography. *Anaesthesia* 53(8):736–743
- Yoshizumi J, Marshall RW, Vickers MD (1991) Effects of low concentrations of cyclopropane and halothane on peak velocity of saccadic eye movements. *Br J Anaesth* 67(6): 735–740
- Brown CH, Green DG (1984) Rod saturation in b-wave of the rat electroretinogram under two different anesthetics. *Vision Res* 24(1):87–90
- Tanskanen P, Kylmä T, Kommonen B, Karhunen U (1996) Propofol influences the electroretinogram to a lesser degree than thiopentone. *Acta Anaesthesiol Scand* 40(4):480–485
- Chaudhary V, Hansen R, Lindgren H, Fulton A (2003) Effects of telazol and nembul on retinal responses. *Doc Ophthalmol* 107(1):45–51

20. Takata I, Adachi E, Chiba J (1982) Influence of fluothane anesthesia on the human ERG. *Nippon Ganka Gakkai Zasshi* 86(12):2166–2171
21. Grinvald A, Frostig RD, Lieke E, Hildesheim R (1988) Optical imaging of neuronal activity. *Physiol Rev* 68(4):1285–1366
22. Tsunoda K, Oguchi Y, Hanazono G, Tanifuji M (2004) Mapping cone- and rod-induced retinal responsiveness in macaque retina by optical imaging. *Invest Ophthalmol Vis Sci* 45(10):3820–3826
23. Abramoff MD, Kwon YH, Ts'o D, Soliz P, Zimmerman B, Pokorny J, Kardon R (2006) Visual stimulus-induced changes in human near-infrared fundus reflectance. *Invest Ophthalmol Vis Sci* 47(2):715–721
24. Jonnal RS, Rha J, Zhang Y, Cense B, Gao W, Miller DT (2007) In vivo functional imaging of human cone photoreceptors. *Opt Express* 15(24):16141–16160
25. Duong TQ, Ngan SC, Ugurbil K, Kim SG (2002) Functional magnetic resonance imaging of the retina. *Invest Ophthalmol Vis Sci* 43(4):1176–1181
26. Cheng H, Nair G, Walker TA, Kim MK, Pardue MT, Thule PM, Olson DE, Duong TQ (2006) Structural and functional MRI reveals multiple retinal layers. *Proc Natl Acad Sci USA* 103(46):17525–17530
27. De La Garza B, Li G, Muir E, Shih YY, Duong TQ (2011) BOLD fMRI of visual stimulation in the rat retina at 11.7 Tesla. *NMR Biomed* 24:188–193
28. Shih YY, De La Garza BH, Muir ER, Rogers WE, Harrison JM, Kiel JW, Duong TQ (2011, in press) Lamina-specific functional MRI of retinal and choroidal responses to visual stimuli. *Invest Ophthalmol Vis Sci*
29. Zhang Y, Peng Q, Kiel JW, Rosende CA, Duong TQ (2011) Magnetic resonance imaging of vascular oxygenation changes during hyperoxia and carbogen challenges in the human retina. *Invest Ophthalmol Vis Sci* 52(1):286–291
30. Nair G, Tanaka Y, Kim M, Olson DE, Thule PM, Pardue MT, Duong TQ (2011) MRI reveals differential regulation of retinal and choroidal blood volumes in rat retina. *Neuroimage* 54:1063–1069
31. Muir ER, Duong TQ (2011) MRI of retinal and choroid blood flow with laminar resolution. *NMR Biomed* 24:216–223
32. Li Y, Cheng H, Duong TQ (2008) Blood-flow magnetic resonance imaging of the retina. *Neuroimage* 39:1744–1751
33. Li Y, Cheng H, Shen Q, Kim M, Thule PM, Olson DE, Pardue MT, Duong TQ (2009) Blood-flow magnetic resonance imaging of retinal degeneration. *Invest Ophthalmol Vis Sci* 50:1824–1830
34. Peng Q, Zhang Y, Oscar San Emeterio Nateras O, van Osch MJP, Duong TQ (2010, in press) Magnetic resonance imaging of blood flow of the human retina. *Magn Reson Med*
35. Bizheva K, Pflug R, Hermann B, Povazay B, Sattmann H, Qiu P, Anger E, Reitsamer H, Popov S, Taylor JR, Unterhuber A, Ahnelt P, Drexler W (2006) Optophysiology: depth-resolved probing of retinal physiology with functional ultrahigh-resolution optical coherence tomography. *Proc Natl Acad Sci USA* 103(13):5066–5071
36. Yao XC, Yamauchi A, Perry B, George JS (2005) Rapid optical coherence tomography and recording functional scattering changes from activated frog retina. *Appl Opt* 44(11):2019–2023
37. Srinivasan VJ, Chen Y, Duker JS, Fujimoto JG (2009) In vivo functional imaging of intrinsic scattering changes in the human retina with high-speed ultrahigh resolution OCT. *Opt Express* 17(5):3861–3877
38. Pardue MT, Phillips MJ, Yin H, Sippy BD, Webb-Wood S, Chow AY, Ball SL (2005) Neuroprotective effect of sub-retinal implants in the RCS rat. *Invest Ophthalmol Vis Sci* 46(2):674–682
39. Pardue MT, Phillips MJ, Yin H, Fernandes A, Cheng Y, Chow AY, Ball SL (2005) Possible sources of neuroprotection following subretinal silicon chip implantation in RCS rats. *J Neural Eng* 2(1):39–47
40. Yoshizumi J, Marshall RW, Sanders LD, Vickers MD (1993) Effects of small concentrations of isoflurane on some psychometric measurements. *Br J Anaesth* 71(6):839–844
41. Wade JG, Stevens WC (1981) Isoflurane: an anesthetic for the eighties? *Anesth Analg* 60(9):666–682
42. Woodward WR, Choi D, Grose J, Malmin B, Hurst S, Pang J, Weleber RG, Pillers DA (2007) Isoflurane is an effective alternative to ketamine/xylazine/acepromazine as an anesthetic agent for the mouse electroretinogram. *Doc Ophthalmol* 115(3):187–201
43. Jonsson Fagerlund M, Dabrowski M, Eriksson LI (2009) Pharmacological characteristics of the inhibition of non-depolarizing neuromuscular blocking agents at human adult muscle nicotinic acetylcholine receptor. *Anesthesiology* 110(6):1244–1252
44. Glover GH, Li TQ, Ress D (2000) Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med* 44(1):162–167
45. Jinks SL, Dominguez CL, Antognini JF (2005) Drastic decrease in isoflurane minimum alveolar concentration and limb movement forces after thoracic spinal cooling and chronic spinal transection in rats. *Anesthesiology* 102(3):624–632
46. Vahle-Hinz C, Detsch O, Hackner C, Kochs E (2007) Corresponding minimum alveolar concentrations of isoflurane and isoflurane/nitrous oxide have divergent effects on thalamic nociceptive signalling. *Br J Anaesth* 98(2):228–235
47. Wixson S, Smiler I (1997) *Anesthesia and Analgesia in Laboratory Animals*. Academic Press, NY
48. Sanders RD, Patel N, Hossain M, Ma D, Maze M (2005) Isoflurane exerts antinociceptive and hypnotic properties at all ages in Fischer rats. *Br J Anaesth* 95(3):393–399
49. Jevtovic-Todorovic V, Benshoff N, Olney JW (2000) Ketamine potentiates cerebrocortical damage induced by the common anaesthetic agent nitrous oxide in adult rats. *Br J Pharmacol* 130(7):1692–1698
50. Pekoe GM, Smith DJ (1982) The involvement of opiate and monoaminergic neuronal systems in the analgesic effects of ketamine. *Pain* 12(1):57–73
51. Peduto VA, Concas A, Santoro G, Biggio G, Gessa GL (1991) Biochemical and electrophysiologic evidence that propofol enhances GABAergic transmission in the rat brain. *Anesthesiology* 75(6):1000–1009
52. Flecknell P (1987) *Laboratory Animal Anesthesia*. Academic Press, London

53. Penn RD, Hagins WA (1969) Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature* 223(5202):201–204
54. Pugh EN Jr, Falsini B, Lyubarsky AL (1998) The origins of the major rod-and cone- driven components of the rodent electroretinogram and the effect of age and light-rearing history on the magnitude of these components. In: TA Williams TP (ed) *Photostasis and related phenomena*. Plenum Press, New York, pp 93–128
55. Hood DC, Birch DG (1990) A quantitative measure of the electrical activity of human rod photoreceptors using electroretinography. *Vis Neurosci* 5(4):379–387
56. Robson JG, Frishman LJ (1998) Dissecting the dark-adapted electroretinogram. *Doc Ophthalmol* 95(3–4):187–215
57. Robson JG, Frishman LJ (1995) Response linearity and kinetics of the cat retina: the bipolar cell component of the dark-adapted electroretinogram. *Vis Neurosci* 12(5):837–850
58. Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA (2000) Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. *J Neurosci* 20(15):5733–5740
59. Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: general considerations. *Experientia* 42(2): 109–114
60. Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 2: cardiovascular system. *Experientia* 42(3):292–297
61. Wachtmeister L (1998) Oscillatory potentials in the retina: what do they reveal. *Prog Retin Eye Res* 17(4):485–521
62. Wenningmann I, Dilger JP (2001) The kinetics of inhibition of nicotinic acetylcholine receptors by (+)-tubocurarine and pancuronium. *Mol Pharmacol* 60(4):790–796
63. Mojumder DK, Wensel TG (2010) Topical mydriatics affect light-evoked retinal responses in anesthetized mice. *Invest Ophthalmol Vis Sci* 51(1):567–576
64. Duong TQ, Muir ER (2009) Magnetic resonance imaging of the retina. *Jpn J Ophthalmol* 53(4):352–367
65. Duong TQ, Pardue MT, Thule PM, Olson DE, Cheng H, Nair G, Li Y, Kim M, Zhang X, Shen Q (2008) Layer-specific anatomical, physiological and functional MRI of the retina. *NMR Biomed* 21(9):978–996
66. TQ Duong (2011) Magnetic resonance imaging of the retina: a brief historical and future perspective. *Saudi J Ophthalmology* 25:137–143