Published in final edited form as:

Doc Ophthalmol. 2017 October ; 135(2): 121–132. doi:10.1007/s10633-017-9597-7.

Isoflurane and ketamine:xylazine differentially affect intraocular pressure-associated scotopic threshold responses in Sprague-Dawley rats

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Abstract

Purpose—Amplitudes of electroretinograms (ERG) are enhanced during acute, moderate elevation of intraocular pressure (IOP) in rats anaesthetised with isoflurane. As anaesthetics alone are known to affect ERG amplitudes, the present study compares the effects of inhalant isoflurane and injected ketamine:xylazine on the scotopic threshold response (STR) in rats with moderate IOP elevation.

Methods—Isoflurane-anaesthetised ($n = 9$) and ketamine:xylazine-anaesthetised ($n = 6$) rats underwent acute unilateral IOP elevation using a vascular loop anterior to the equator of the right eye. STRs to a luminance series (sub-threshold to -3.04 log scotopic cd-s/m²) were recorded from each eye of Sprague-Dawley rats before, during, and after IOP elevation.

Results—Positive STR (pSTR) amplitudes for all conditions were significantly smaller ($p=0.0001$) for isoflurane- than for ketamine:xylazine-anaesthetised rats. In addition,

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DISCLOSURES

Funding: This study was funded in parts by Natural Sciences and Engineering Research Council (NSERC) Discovery Grants (VC; KB), the University of Waterloo CIHR Research Incentive Fund (KB, VC, KMJ), the University of Waterloo Propel Centre Disease Prevention Initiative Seed Grant (VC, KB, KMJ), Canadian Foundation for Innovation Leaders Opportunity Fund (VC), Joseph Ellis Family Glaucoma Research Fund (KMJ), William Black Glaucoma Research Fund (KMJ), NIH 6P30EY008126-28 to Vanderbilt Vision Research Center (KMJ), and an Unrestricted Departmental Grant from Research to Prevent Blindness, Inc., N.Y. to the Vanderbilt Eye Institute (KMJ). Sponsors had no role in the design or conduct of this research.

Conflict of interest: AG presented some of this work at the ARVO 2016 Meeting and was supported with travel grants from NSERC (VC), the School of Optometry and Vision Science and the Graduate Studies Office at the University of Waterloo. VC is a co-applicant on a patent describing work supported by the NSERC Grant but not related to the subject matter. The authors certify that they have no other affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

ketamine:xylazine was associated with a progressive increase in pSTR amplitudes over time ($p=0.0028$). IOP elevation was associated with an increase in pSTR amplitude (both anaesthetics $p<0.0001$). The absolute interocular differences in IOP-associated enhancement of pSTR amplitudes for ketamine:xylazine and isoflurane were similar ($66.3 \pm 35.5 \mu\text{V}$ vs $54.2 \pm 24.1 \mu\text{V}$, respectively). However, the fold-increase in amplitude during IOP elevation was significantly higher in the isoflurane- than in the ketamine:xylazine-anaesthetised rats ($16.8 \pm 29.7x$ vs $2.1 \pm 2.7x$, respectively, $p=0.0004$).

Conclusions—The anaesthetics differentially affect the STRs in the rat model with markedly reduced amplitudes with isoflurane compared to ketamine:xylazine. However, the IOP-associated enhancement is of similar absolute magnitude for the two anaesthetics, suggesting that IOP stress and anaesthetic effects operate on separate retinal mechanisms.

Keywords

anaesthetics; isoflurane; ketamine:xylazine; intraocular pressure elevation; rat; electroretinogram (ERG); scotopic threshold response (STR)

INTRODUCTION

Electroretinograms (ERGs) provide a relatively non-invasive compound measure of retinal cell function [1]. The scotopic threshold response (STR) is the ERG waveform generated in dark-adapted eyes by weak light stimulation below the threshold for the a- and b-waves, which reflects primarily the function of third-order neurons [2], such as the retinal ganglion cells (RGCs) in rodents [3–6] and in humans [7]. The STR has both a negative and a positive component. The nSTR likely reflects primarily amacrine cell function [8,9], while the more robust positive component of the STR (pSTR), which has a higher threshold and shorter latency than the nSTRs [10], has been postulated to reflect primarily retinal ganglion cell function [8,9].

Studies showing that STRs are affected in conditions that compromise RGC function support the idea that STRs may be used to assess RGC function [3,11,12]. Acute ischaemic intraocular pressure (IOP) levels reduce or extinguish STRs [13–15] and other ERG waveforms [13,15]. However, acute, IOP elevation to moderate levels around 35 mmHg is associated with enhanced ERG and STR waveforms, which are extinguished at ischaemic levels (80 mmHg) [16]. These enhanced STR and ERG waveforms were recorded while rats were anaesthetised with isoflurane. Most STR studies based on animal models have used the injectable general anaesthetics ketamine:xylazine [3,4,17–20]. However, no information is available about how the two anaesthetics might differentially affect ERGs at levels used to record STRs.

The present study was undertaken to compare the effects of isoflurane to those of ketamine:xylazine on the STRs in rats and their influence on the STR enhancement associated with acute, moderately-elevated IOP.

METHODS AND MATERIALS

Animals

All procedures were carried out in accordance with the Canadian Council of Animal Care (University of Waterloo) and/or the Institutional Animal Care and Use Committee (Vanderbilt University), and conformed to the ARVO statement for the use of animals. Eleven-week old (~300 g) male Sprague-Dawley rats (Harlan Labs, Indianapolis, Indiana) were fed *ad libitum*. The rats were dark-adapted for at least 12 hours before being anaesthetised with either 3% isoflurane in oxygen (n=9), delivered via a nose cone attached to a head-rest, or with 0.3 ml of a cocktail of 40.4 mg/kg ketamine and 4.0 mg/kg xylazine (n=6), via intraperitoneal injection. Rats that were anaesthetised with isoflurane were maintained between 2 to 3% isoflurane in oxygen for the entirety of the experiment, while those anaesthetised with ketamine:xylazine were re-injected with 0.20 ml of the same dosage every 40 ± 5 minutes to maintain anaesthesia. As the procedures took about 2 hours, ketamine:xylazine-anaesthetised animals were typically injected a total of 3 or 4 times during the experimental period; these animals usually recovered from the anaesthesia about 30 minutes after the end of the procedure.

STR recordings

All preparations and interventions were conducted under dim red illumination (631 nm, less than 10.9 lux) to minimise light adaptation. Rats were placed onto a water-heated stage (38°C, Gaymar, TP650, HHP05, Orchard Park, NY) located within a light-excluding chamber. One drop of the topical anaesthetic proparacaine hydrochloride (Alcaine: #1001600, Alcon, Mississauga, ON, Canada) was instilled in each eye, followed by one drop of 0.5% tropicamide (mydriacyl: Alcon, Mississauga, ON, Canada) to dilate the pupils. Custom-made monopolar silver-chloride circular electrodes were placed onto the limbus of each eye so that the electrodes were touching but not pressing onto the eyes [21]. Reference electrodes were placed under the skin just above the ears [22] such that the tip of electrode was ~2.5 mm behind the lateral canthi of each eye. The ground electrode was placed at the back of the head. Hydration of the corneas was maintained using Refresh® Celluvisc drops (Allergan, Parsippany, NJ) as needed. The stimuli for the STRs were 4 ms full-field flashes of white light (from light-emitting diodes with an equivalent colour temperature of 6500 °K) delivered binocularly using commercial handheld mini ganzfeld stimulators (D213 Espion Colorburst®, Diagnosys LLC, Lowell, MA) directly in front of each eye within a light-proof chamber. The mini ganzfeld stimulators were calibrated when acquired and verified with internal calibration software. Each luminance step was separated by a 2-second dark interval; responses to 40 to 60 flashes per luminance step were filtered with a band pass of 0.312 to 300 Hz and averaged over a 500 ms epoch. The time-integrated luminance of the flash stimuli increased in 0.2 log steps, starting from $-6.64 \log$ scotopic $\text{cd}\cdot\text{s}/\text{m}^2$, and ending at $-3.04 \log$ scotopic $\text{cd}\cdot\text{s}/\text{m}^2$.

IOP Elevation

STR luminance series were recorded before, during and after the physiological stress of moderate IOP elevation. IOP elevation was achieved by placing a vascular loop (Sentinal Loops; Sherwood-Davis and Geck, St. Louis, MO) around the right eye anterior to the

equator, as per Joos *et al.* [23]. IOPs were measured throughout the experiment (10 minutes prior to each intervention or recording) using a rebound tonometer (average of 5 readings; Icare® Tonolab, Icare Finland Oy, Helsinki, Finland); the 10 minute interval was used to re-adapt the rats to complete darkness. The loop was adjusted when necessary to achieve the target IOP of 35 mmHg since the intent was to produce moderate IOP elevation rather than non-perfusion of the eye. The loop was not placed around the left eye as it was used as a control for mechanical stress. STRs were recorded binocularly prior to any intervention and then beginning 45 minutes into the one-hour loop-wear procedure, as well as starting 30 min or 45 min after the loop removal for the isoflurane-anaesthetised animals and 45 minutes after loop removal for the ketamine:xylazine-injected animals.

Data Analysis

The peak amplitudes of the pSTRs were measured if the peak positive change in voltage from the baseline voltage to the first peak was above the pre-flash noise level. Implicit times were measured from the midpoint of the 4 ms stimulus flash to the peak amplitude.

A priori analyses of the effects on the pSTR amplitudes and implicit times for each anaesthetised set of rats were carried out using repeated measures analysis of variance (ANOVA) tests with Greenhouse-Geisser epsilon corrections where necessary (epsilon values <0.75). Post-loop STR amplitudes and implicit times collected in the isoflurane-anaesthetised rats at 30 min after the loop removal were not significantly different from those collected after 45 min ($p=0.1877$ and $p=0.7604$, respectively), therefore these data were pooled prior to further analysis. To compare effects of anaesthetics and effects of IOP for both absolute and relative differences in STRs between treated and control eyes, mixed model ANOVAs were used. Bonferroni-corrected multiple comparison tests or Tukey tests were used for all post-hoc testing of dependent and independent data, respectively. Values were considered significant for $p<0.05$. All means are reported with the standard deviation (SD) unless otherwise noted.

RESULTS

Comparison of anaesthetic effects for baseline STRs

Peak amplitudes and implicit times were measured from STR recordings elicited with flash stimuli greater than $-4.24 \log$ scotopic $\text{cd}\cdot\text{s}/\text{m}^2$ as per Choh *et al.*, [16]. At baseline (prior to any intervention) for each anaesthetic, the pSTR amplitudes for the control and treated eyes were similar ($p>0.9999$ for all luminance steps; Fig. 1). Higher flash luminance levels were strongly associated with larger pSTR amplitudes for ketamine:xylazine anaesthetised animals ($p<0.0001$), but not significantly for isoflurane-anaesthetised animals ($p=0.0741$). For all luminance levels greater than $-4.24 \log$ scotopic $\text{cd}\cdot\text{s}/\text{m}^2$, the ketamine:xylazine-associated pSTR amplitudes were substantially larger than those associated with isoflurane ($p=0.0377$; Fig. 1b).

Effects of elevated IOP on isoflurane-anaesthetised animals (n=9)

Increasing the IOP to the targeted 35 mmHg led to a striking enhancement of the pSTR amplitudes in isoflurane-anaesthetised animals (Figs. 2a and 2b), with higher luminance

levels evoking larger amplitudes (Fig. 2c). In treated eyes, the average IOP across luminance levels during the loop procedure was 33.3 ± 3.3 (SD) mmHg, about 25 mmHg higher than the pre-loop baseline IOP of 8.4 ± 2.0 , and the post-loop IOP level of 8.7 ± 2.6 mmHg (Fig. 2b). In these eyes, IOPs prior to and following loop-wear were not different ($p>0.9999$), while those during loop-wear were different from both pre- and post-loop conditions ($p<0.0001$ for both comparisons). IOPs in the fellow (control) eyes did not change as a function of loop-wear in the opposite eye ($p>0.9999$ for all comparisons).

The pSTR amplitudes in the treated eyes during loop-wear (mean $\pm$ S.D. across luminance levels: 61.2 ± 24.8 μ V) were markedly enhanced compared to those before loop-wear (6.4 ± 2.6 μ V; $p<0.0001$) and those after loop removal (10.1 ± 5.1 μ V; $p<0.0001$) (Fig. 2c). pSTR amplitudes before and after loop-wear were not significantly different ($p>0.9999$ for all stimuli). The pSTR increase was limited to the treated eye; no differences in pSTR amplitudes were detected in the control (untreated eyes) as a function of loop-wear ($p>0.9999$ for all comparisons), indicating an absence of an IOP-associated fellow-eye effect. Pre- and post-loop pSTR amplitudes were not different between the treated and control eyes prior to loop placement (6.4 ± 2.6 μ V vs 6.3 ± 3.4 μ V, respectively, $p>0.9999$) or post-loop placement (10.1 ± 5.1 μ V vs 8.2 ± 2.2 μ V, respectively, $p>0.9999$). However, during loop-wear, the pSTR amplitudes were markedly higher in the treated eyes than in the control eyes (61.2 ± 24.8 μ V vs. 7.0 ± 2.4 μ V, respectively; $p<0.0001$; Fig. 2c). Overall implicit times were greater in treated eyes (105.0 ± 21.6 ms) compared to those in control eyes (101.0 ± 23.2 ms; $p=0.0125$; Table 1), the difference in the STR implicit times as a function of loop-wear was not significant ($p=0.0742$). Implicit times did not change as a function of the strength of the stimulus flash ($p=0.4347$).

Effects of elevated IOPs in ketamine:xylazine-anaesthetised animals (n=6)

In general, the pattern for ketamine:xylazine-injected animals was similar to that of the isoflurane-anaesthetised rats (Fig. 3a). Elevating IOP to levels similar to those for the isoflurane-anaesthetised rats (Fig. 3b) led to enhanced pSTR amplitudes that increased with increasing luminance levels (Fig 3c). The mean IOP during the loop procedure in the ketamine:xylazine-anaesthetised animals was 32.5 ± 3.3 mmHg, which was about 26 mmHg greater than the baseline (6.7 ± 1.4 mmHg) and post-loop (6.4 ± 2.6 mmHg) readings (Fig. 3b). For the treated eyes, IOPs prior to and following loop-wear were not different ($p>0.9999$), while those during loop-wear were different from both pre- and post-loop conditions ($p<0.0001$ for both). IOPs in control eyes did not change as a function of loop-wear ($p>0.9999$ for all comparisons).

For ketamine:xylazine-injected animals, pSTR amplitudes and implicit times were measured at the same stimulus levels (greater than -4.24 log scotopic $\text{cd}\cdot\text{s}/\text{m}^2$) as for the isoflurane-anaesthetised rats. pSTR amplitudes for each eye increased as the stimulus flash luminance increased ($p<0.0001$). Moderate, acute IOP elevation using the loop led to increased pSTR amplitudes in the treated eyes ($p=0.0069$); the mean pSTR amplitude across luminance levels increased from the baseline value of 58.3 ± 26.6 μ V to 158.5 ± 37.3 μ V in the treated eyes ($p<0.0001$). However, unlike the case for isoflurane-anaesthetised animals, the pSTR amplitudes did not drop significantly after the loop was removed ($p=0.1347$; compare 158.5

$\pm 37.3 \mu\text{V}$ vs $126.4 \pm 30.7 \mu\text{V}$, respectively). In fact, post-loop pSTR amplitudes were significantly greater than the pre-loop amplitudes ($p=0.0007$; compare $126.4 \pm 30.7 \mu\text{V}$ vs $58.3 \pm 26.6 \mu\text{V}$, respectively). The higher post-loop values are consistent with systemic changes to the ketamine:xylazine-anaesthetised animals affecting the ERGs; control eyes also showed a significantly higher pSTR amplitude at the end of the experiment compared to the start of the experiment ($p=0.0028$), with values increasing from baseline values of $58.4 \pm 21.3 \mu\text{V}$ to $115.5 \pm 27.0 \mu\text{V}$. The baseline amplitudes in both the treated and control eyes were similar ($p>0.9999$, $58.3 \pm 26.6 \mu\text{V}$ vs $58.4 \pm 21.3 \mu\text{V}$, respectively), as were the amplitudes at the end of the experiment ($p>0.9999$; $126.4 \pm 30.7 \mu\text{V}$ vs $115.5 \pm 27.0 \mu\text{V}$, respectively). Only during loop-wear were pSTR amplitudes significantly greater in the treated eyes compared to their controls ($p=0.0008$; $158.5 \pm 37.3 \mu\text{V}$ vs $92.2 \pm 13.2 \mu\text{V}$, respectively).

Implicit times for the larger pSTRs in the ketamine:xylazine-anaesthetised rats were less variable than for the smaller responses in the isoflurane anaesthetised rats (Table 2). Increasing the flash luminance elicited shorter implicit times ($p=0.0119$; data not shown) and the use of the loop led to different implicit times between the eyes ($p=0.0021$). Specifically, in the treated eyes, implicit times during loop-wear ($119.7 \pm 7.0 \text{ ms}$) were longer than the baseline ($109.5 \pm 5.0 \text{ ms}$; $p=0.0001$) and the post-loop values ($105.7 \pm 6.9 \text{ ms}$; $p<0.0001$). In the control eyes, the pSTR implicit times prior to and during loop-wear were similar (compare $109.2 \pm 4.6 \text{ ms}$ vs $110.1 \pm 5.5 \text{ ms}$, respectively; $p>0.9999$). However, post-loop implicit times ($105.4 \pm 7.3 \text{ ms}$) were significantly shorter than the loop-on values ($p=0.0280$; $110.1 \pm 5.5 \text{ ms}$), although not significantly shorter than the pre-loop values ($p=0.0939$; $109.2 \pm 4.6 \text{ ms}$). Comparing treated and control eyes, the pSTR implicit times were neither statistically different prior to loop placement ($109.5 \pm 5.0 \text{ ms}$ vs $109.2 \pm 4.6 \text{ ms}$, $p>0.9999$) nor after removing the loop ($105.7 \pm 6.9 \text{ ms}$ vs $105.4 \pm 7.3 \text{ ms}$, $p>0.9999$). However during IOP elevation, pSTR implicit times were longer in the treated eyes than in the control eyes ($119.7 \pm 7.0 \text{ ms}$ vs. $110.1 \pm 5.5 \text{ ms}$, respectively; $p=0.0001$).

Comparison of STRs in isoflurane vs ketamine:xylazine-anaesthetised animals

Comparisons between the isoflurane- ($n=9$) and ketamine:xylazine-anaesthetised ($n=6$) animals showed that pSTR amplitudes were greater in the latter group for all loop conditions ($p=0.0001$; Fig. 4a). The absolute difference between the treated and control eyes (interocular difference: amplitude in treated eye minus amplitude in control eye), which accounts for the systemic increase in pSTR amplitudes in the ketamine:xylazine-anaesthetised animals, did not differ based on the anaesthetic used ($p=0.5741$; compare ketamine:xylazine- vs isoflurane-anaesthetised interocular difference for loop-wear condition: $66.3 \pm 35.5 \mu\text{V}$ vs $54.2 \pm 24.1 \mu\text{V}$, respectively; Fig 4b). However, the fold-change in amplitudes (fold-change equals amplitude in treated eye divided by amplitude in control eye) was significantly greater, by about a factor of 10, during loop-wear ($p=0.0004$; Fig 4c) for the isoflurane-anaesthetised rats (fold change: $16.8 \pm 29.7\text{x}$) compared to the ketamine:xylazine-anaesthetised rats (fold change: $2.1 \pm 2.7\text{x}$). The anaesthetics were not associated with any differential effects on implicit time ($p=0.7247$).

DISCUSSION

The results clearly indicate that isoflurane has a strong effect on the STR amplitudes in rats, which includes obscuring the pSTR threshold for isoflurane-anaesthetised rats in the absence of a loop, especially in the presence of noise. In the present study, high frequencies were retained in the waveforms using standard ERG filtering with an analogue band pass of 0.312 to 300 Hz [24], resulting in more high frequency noise than in previous studies that filtered frequencies above 30 Hz [3,13,16,25]. While the threshold for isoflurane-anaesthetised rats prior to loop-wear was not as distinct, a clear threshold was observed for these rats during loop-wear (Fig. 2c), and the value of the threshold matched that of our previous study, $-4.24 \log \text{cd}\cdot\text{s}/\text{m}^2$ [16]. This threshold value was also observed for ketamine:xylazine-anaesthetised rats with and without a loop in the present study (Figs. 1, 3c). These data suggest that the threshold value was unaffected by whether IOP was elevated or not in the eyes and are also similar to the IOP-independent threshold values observed for isoflurane-anaesthetised rats in our previous experiment [16]. In addition to matching the threshold for the pSTRs in our previous study, the pSTRs thresholds in the present study fall within the luminance range used by other investigators [3,5,26].

The observations of higher pSTR amplitudes with ketamine:xylazine than with isoflurane are not surprising given the similar effects of these anaesthetics on other ERG components [27,28]. Nair and colleagues [28] showed that both the ERG a-wave and b-wave amplitudes were smaller when rats were anaesthetised with isoflurane compared to those in rats anaesthetised by ketamine:xylazine, and in our study, isoflurane also suppressed pSTR amplitudes relative to those recorded with ketamine:xylazine (Fig. 4a). An association between implicit times and pSTR amplitudes was not detected with isoflurane, most likely due to the very small pSTRs associated with high variance in implicit time measurements. The implicit times for the pSTRs in the present study are shorter than those reported in earlier studies [3,16,25]. Such differences are expected because removing high frequencies with analogue filters induces phase shifts (delays) in implicit times [29,30].

While the pSTRs in the ketamine:xylazine-anaesthetised rats would at first seem to be ideal for detecting changes in unmanipulated eyes, ketamine:xylazine is also associated with progressive enhancement of pSTR amplitudes over time that includes the fellow eyes, a result that has also been shown in at least one other study [15]. Thus, the use of isoflurane would not be ideal for experiments designed to characterise STR dynamics, as both baseline implicit times and baseline amplitudes need to be robust. However, the use of ketamine:xylazine may not be ideal for experiments where consistent pSTR amplitudes over time are important in the control eyes or control animals. The systematic ketamine:xylazine-associated increase would be particularly problematic in situations where the treated and control eyes cannot be measured simultaneously to account for the trend, or if the durations of the procedures for treated and control animals differ.

The two methods of anaesthesia did not lead to a difference in the absolute IOP-associated enhancement of pSTR amplitudes (Fig 4b), while the relative fraction or percentage change was very different (Fig 4c). This dissimilarity indicates that the mechanism(s) for the anaesthetic effect and IOP-associated effect on the pSTR differ and are not proportional. The

optimal choice of anaesthetic may depend on the question to be addressed by the intended experiment.

The exact mechanism for isoflurane-mediated suppression of retinal activity is unknown. In their work showing that the amplitudes of multifocal ERGs in pigs are suppressed by isoflurane compared to ketamine, Lalonde and colleagues [31] suggested that isoflurane might act to suppress retinal ganglion cell function by inhibiting retinal synaptic activity upstream to the RGCs. Similarly, it remains unclear what mechanism mediates the IOP-independent ketamine:xylazine-induced progressive increase in pSTR amplitudes (Fig. 3c). One possible mechanism involves the indirect effects of the ketamine:xylazine cocktail. It was previously shown that 15 minutes following a single intraperitoneal injection of ketamine:xylazine, not only did blood glucose levels in C57/BL6J mice more than double, but they also continued to rise throughout the time course of a 60 minute experiment [32]. Several investigators have shown independently that hyperglycemia is associated with enhanced retinal responses, and hypoglycemia with reduced responses [33–35]. Alternatively, or additionally, ketamine and xylazine could act directly or indirectly via the NMDA and adrenergic receptors to which they respectively bind [32,36,37]. Xylazine is an α_2 -adrenergic receptor agonist; Jarkman and Bragadottir [38] observed that b-wave amplitudes were enhanced with clonidine, another α_2 -adrenergic receptor agonist. We do not believe that the progressive increase in amplitudes were due to changes in the depth of anaesthesia of the rats during the procedure, since we maintained the anaesthetic plane of the animals by injecting supplemental doses of ketamine:xylazine.

Our results show that mechanically stressing the eye to produce acute, moderate IOP elevation is associated with increases in STR signals that were robust both for the ketamine:xylazine cocktail and for isoflurane. The elevated IOP was associated with markedly enhanced pSTR amplitudes compared to baseline values in both anaesthetic groups. These data, at first, appear to be contradictory to other experiments involving STRs and IOP changes, which tend to show decreases in STR amplitudes [13,22,25]. However, the STRs in the present study were recorded within a relatively short time, less than an hour of elevated IOP, and represent an acute stress response, rather than a delayed and/or chronic stress response associated with cell death [17,25,39]. Previous work by our group indicates that acute IOP elevation to around 35 mmHg enhances the amplitudes of all scotopic ERG components, including the a-wave, b-wave and oscillatory potentials in rats anaesthetised with isoflurane [16]. Supra-normal amplitudes of various ERG waveforms have been also observed for chronic studies with moderate IOP elevation; Frishman *et al.* [40] and Nork *et al.* [41] have noted elevated IOP-associated increases in multi-focal ERG first-order kernel response amplitudes that appear to be independent of ganglion cell loss [42].

The IOP elevation was maintained at 33–40 mmHg in our experiments, whereas some previous studies included IOP levels that were higher and were therefore ischaemic [14,19,22,43,44]. We also have reported that using the loop at ischaemic IOP levels (80 mmHg) abolishes STRs [16]. Other studies used cannulation to increase the IOP [13–15,45]; figures from studies by Tsai and colleagues [15] and Bui *et al.* [13] show small relative increases in pSTR amplitudes in eyes with IOP-elevated to around 30 mmHg using a cannula in ketamine:xylazine-anaesthetised rats.

It remains unclear what mechanism exists to enhance STRs with moderate, acute elevation of IOPs. The time course for the increase in STR amplitudes was not measured to avoid light-adapting the eyes. We considered the possibility that the vascular loop could mechanically alter the light distribution over the retina by changing the ocular shape, even at these modest levels of pressure. However, retinal illumination is almost independent of ocular dimensions when using Ganzfeld stimulation with dilated pupils. Furthermore, ERGs are smaller in myopic eyes [46], thus any elongation of the eye would not account for markedly increased amplitudes. Factors that are released upon mechanically stressing, but not damaging, the eye include nitric oxide [47] and adenosine [48,49], both of which have been shown to enhance ERG a- and b-waves (for a fuller discussion see Choh [16]). Moreover, the IOP-associated enhancement of the ERG amplitude is reversible, with amplitudes reverting to levels similar to those prior to the addition of the exogenously added factors [47,48]. The decrease in STR amplitudes during the post-loop condition in our study is consistent with a system that is reversible.

In summary, ketamine:xylazine was associated with larger STR amplitudes and with longer implicit times, and was also associated with a systematic increase in STR amplitudes over time. Responses for mechanically stressed eyes in rats with moderately elevated IOP under isoflurane were markedly enhanced when analysing percentage changes, but the absolute enhancement of STRs in eyes with elevated IOP were similar, irrespective of the anaesthetic used. Together, the results suggest that IOP stress and anaesthetic effects operate on separate retinal mechanisms.

Acknowledgments

The authors acknowledge Shahrokh Khoshshobhat for constructing the dark-adaptation box and assisting with the development of the STR protocol and Nancy Gibson for assistance with animal handling. Thank you Jin Shen and Ratna Prasad for technical support while conducting experiments at Vanderbilt. This work was funded in part by Natural Sciences and Engineering Research Council (NSERC) Discovery Grants, the University of Waterloo CIHR Research Incentive Fund, the University of Waterloo Propel Centre Disease Prevention Initiative Seed Grant, Joseph Ellis Family Glaucoma Research Fund, William Black Glaucoma Research Fund, NIH 6P30EY008126-28 to Vanderbilt Vision Research Center, and an Unrestricted Departmental Grant from Research to Prevent Blindness, Inc., N.Y. to the Vanderbilt Eye Institute. Funding sources had no involvement in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

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Fig. 1.

Comparison of anaesthetics on the baseline scotopic threshold responses (STRs) at various luminance levels. (a) Sample series from a single rat showing positive STRs (pSTRs) at baseline in the eye to receive treatment (black lines) and its fellow control eye (grey lines). (b) Mean pSTR amplitudes $\pm$ SEM for rats anaesthetised with isoflurane (circles $n=9$) and ketamine:xylazine (triangles $n=6$) for eyes to receive treatment (filled symbols) and their fellow controls (empty symbols)

Fig. 2.

Effects of IOP elevation on pSTRs of isoflurane-anaesthetised rats. (a) Sample pSTRs at $-4.04 \log$ scotopic $\text{cd}\cdot\text{s}/\text{m}^2$ from a treated eye (black lines) and a control eye (grey lines) of a rat before, during and after IOP elevation. (b) Mean IOPs $\pm$ SEM and (c) pSTR amplitudes $\pm$ SEM from treated eyes (filled symbols) and control eyes (empty symbols) during the various stages of loop-wear. Asterisks (*) denote significant differences ($P < 0.05$) relative to the respective pre-loop condition within the same eye. Daggers (†) represent differences ($P < 0.05$) between eyes for the specific loop condition

Fig. 3. Effects of IOP elevation on pSTRs of ketamine:xylazine-anaesthetised rats. (a) Sample pSTRs at $-4.04 \log \text{scotopic cd}\cdot\text{s}/\text{m}^2$ from a treated eye (black lines) and a control eyes (grey lines) of a rat before, during and after IOP elevation. (b) Mean IOPs $\pm$ SEM and (c) pSTR amplitudes $\pm$ SEM from treated eyes (filled symbols) and control eyes (empty symbols) during the various stages of loop-wear. Asterisks (*) denote significant differences ($P < 0.05$) relative to the respective pre-loop condition within the same eye. Daggers (†) represent differences ($P < 0.05$) between eyes for the specific loop condition

Fig. 4.

Comparisons of anaesthetics on IOP-associated pSTRs. (a) Average positive STRs $\pm$ SEM in ketamine:xylazine-anaesthetised animals (kx; triangles), across luminance levels were generally higher than in isoflurane-anaesthetised animals (iso; circles). Positive STR amplitudes in treated eyes (filled symbols) were greater than those in control eyes (empty symbols) during loop-wear for both anaesthetics. The average absolute difference between the eyes was not different between the anaesthetics (b) but the fold-increase between the eyes was much greater during loop-wear for rats anaesthetised with isoflurane (c). Asterisks (*) denote significant differences ($P < 0.05$) relative to the respective pre-loop condition within the same eye. Daggers (†) represent differences ($P < 0.05$) between eyes for the specific loop condition. Double daggers (‡) denote significant differences ($P < 0.05$) between anaesthetics for the specific eye and loop condition

Table 1

Mean implicit times of scotopic threshold responses to luminance steps of -3.04 to -4.24 log scotopic $\text{cd}\cdot\text{s}/\text{m}^2$ for isoflurane-anaesthetised rats ($n=9$) in each eye as a function of the IOP raising procedure.

IOP Procedure	Treated Eye $\pm$ SD	Control Eye $\pm$ SD
Pre-loop	102.1 ± 9.3 ms	100.5 ± 8.9 ms
During Loop	112.4 ± 9.7 ms	102.7 ± 11.4 ms
Post-loop	100.5 ± 11.5 ms	99.9 ± 14.3 ms

Overall, implicit times in treated eyes were longer than in control eyes ($P = 0.0125$).

Table 2

Mean implicit times of scotopic threshold responses to luminance steps of -3.04 to -4.24 log scotopic $\text{cd}\cdot\text{s}/\text{m}^2$ for ketamine:xyalzine-anaesthetised rats ($n=6$) in each eye as a function of the IOP raising procedure.

IOP Procedure	Treated Eye $\pm$ SD	Control Eye $\pm$ SD
Pre-loop	109.5 ± 5.0 ms	109.2 ± 4.6 ms
During Loop	119.7 ± 7.0 ms *†	110.1 ± 5.5 ms
Post-loop	105.7 ± 6.9 ms	105.4 ± 7.3 ms

Asterisks (*) denote significant differences ($P < 0.05$) relative to the respective pre-loop condition. Daggers (†) represent differences ($P < 0.05$) between eyes for the specific loop condition. For more comparisons, please see text.