Interaction of anaesthetic drugs and UV-B irradiation in the anterior segment of the rat eye

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ABSTRACT.

Purpose: To determine the impact of anaesthesia on acute transient cataractogenesis and ultraviolet radiation (UVR)-induced cataractogenesis.

Methods: Sprague-Dawley rats were anaesthetized with pentobarbital, which caused almost full eyelid closure, or xylazine/ketamine, which caused eyelid retraction and proptosis. The eyelids of one eye were kept open with either a suture or adhesive tape, or both. The other eye was kept closed with either a suture or tape. Cataract was graded clinically and quantified *in vitro* as intensity of forward light scattering. In two UVR experiments, anaesthetized rats were irradiated unilaterally with 5 kJ/m² UVR-B 300 nm for 15 mins. The difference between the two UVR experiments was the degree of proptosis in the pentobarbital group. Corneal drying was judged clinically with a grading scale.

Results: Within 60 mins of anaesthesia induction in the first experiment, almost all lenses in open eyes developed cataract, whereas all lenses in closed eyes remained clear. In the first UVR experiment the lens light scattering was significantly higher in the xylazine/ketamine group. In the second UVR experiment the pentobarbital group was treated to achieve proptosis similar to that in the xylazine/ketamine group, which led to a smaller difference in lens light scattering between the two anaesthesia groups. Lens light scattering in the pentobarbital groups was significantly higher with forced proptosis than without prominent proptosis.

Conclusions: Xylazine/ketamine anaesthesia facilitates the development of UVR-induced cataract, whereas pentobarbital anaesthesia does not. Xylazine/ketamine anaesthesia induces more proptosis and therefore leads to increased exposure of the cornea and, secondarily, the lens.

Key words: xylazine – ketamine – pentobarbital – ultraviolet radiation – lens – reversible – cataract – light scattering – rat

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Introduction

The current study aimed to study the effect of anaesthesia on crystalline lens clarity.

Cataract is the leading cause of blindness in the world (World Health Organization 2004). Cataract is a multifactorial disease characterized by loss of vision due to the scattering of light in the lens. Ageing, diabetes, exposure to solar ultraviolet radiation (UVR), steroid treatment and smoking are all factors that have been epidemiologically associated with cataract.

In order to increase understanding of the mechanism of UVR-induced cataract, it is necessary to expose experimental animals to UVR. In experiments on the immediate development of cataract after experimental exposure to UVR, anaesthetics are used to immobilize animals during UVR exposure (Söderberg 1988; Söderberg 1990a). Two common types of anaesthetic have been used by our group and others: pentobarbital (Söderberg 1990b) and a combination of xylazine and ketamine (Michael 2000; Löfgren 2001; Ayala 2005; Dong 2005). Traditionally, barbiturates, such as pentobarbital, have been the anaesthetics of choice for laboratory rodents. However, pentobarbital has a narrow therapeutic dose range and increased mortality in the dose range needed to achieve efficient anaesthesia (Brown & Ferner 1985). The combination of xylazine and ketamine began to be used as an alternative to barbiturate anaesthesia in veterinary and laboratory work in the late 1970s. Xylazine itself is an analgesic, but it is mainly used for its sedative and muscle relaxant effects when applied in combination with the dissociate anaesthetic ketamine.

Acute reversible cataract was described by Fraunfelder & Burns (1970) as cataract of rapid onset,

usually appearing within 1 hour, and clearing after a couple of hours, whether or not the initiating stimulus was continued. A series of experiments showed that cataract could be induced if the evelids were held open, regardless of the technique for holding them open and whether or not the animal was anaesthetized (Fraunfelder & Burns 1962, 1966, 1970; Hanna & Fraunfelder 1971). In all cases, closure of the eyelids prevented the development of lens opacity. The use of xylazine/ketamine in ophthalmic research was cautioned by Calderone et al. (1986), who had observed the development of acute reversible cataract in rats and mice anaesthetized with xylazine/ketamine or xylazine alone, even after topical administration of xylazine. They concluded that the evaporation of water from the cornea and the subsequent hyperosmolarity of the aqueous humour caused the lens opacification. Pentobarbital in combination with ketamine was shown not to induce cataract and was thus suggested as a safe anaesthetic for ophthalmic research (Kufoy et al. 1989).

In the present study, we wanted to complement earlier qualitative experiments with quantitative measurements of the degree of cataract after xylazine/ketamine and pentobarbital anaesthesia, combined with varying eyelid positions. Further, we wanted to quantify the effect of pentobarbital and xylazine/ketamine, respectively, on cataract development after *in vivo* exposure of the eye to UVR.

Materials and Methods

Animals and anaesthetic drugs

The experiments were approved by the Stockholm Animal Experiments Ethical Committee and conformed with the ARVO statement on the use of animals in vision and ophthalmic research. A total of 142 6-weekold female albino Sprague-Dawley (B & K Universal AB, Stockholm, Sweden) rats were used in three experiments. Two rats died during pentobarbital anaesthesia and were replaced. The rats were anaesthetized with an intraperitoneal injection of either 40 mg/kg pentobarbital (Pentobarbital; Apoteksbolaget, Stockholm, Sweden), or a mixture of 80 mg/kg

ketamine (Ketalar; Parke Davis, Stockholm, Sweden) and 10 mg/kg xylazine (Rompun; Bayer, Stockholm, Sweden).

Experiment 1: anaesthetic and eyelid position versus anterior segment reaction, eyelids sutured

In this experiment, we determined qualitatively and quantitatively the cataractogenic effect of anaesthetics in closed versus open eyes. The animals were randomized into two anaesthetized groups and one non-anaesthetized control group of 20 rats each (Fig. 1).

To facilitate slit-lamp inspection of the lenses, the mydriatic tropicamide Mydriacyl (5 mg/ml) (Alcon, Stockholm, Sweden) was instilled in both eves a few minutes after the drug injection. Xylazine/ketamine anaesthesia induces evelid retraction and proptosis (eve protrusion) in rats and mice, whereas pentobarbital induces almost complete eyelid closure. A nylon suture (5-0 Ethilon; Ethicon, Stockholm, Sweden) was used to close one eve in all rats in the two drug groups to achieve similar eyelid closure. In the xylazine/ketamine group, the nonsutured eye was naturally open. In the pentobarbital group, the non-sutured eye was held open by a suture in the upper eyelid and adhesive tape on the

lower. The tape pulled the lower eyelid down without causing added pressure on the eye.

The eyes were inspected with a slitlamp microscope at 30 mins (open eyes only) and 60 mins (both eyes) after the induction of anaesthesia. The stage of lens opacity was determined *in vivo* according to the qualitative method of Kufoy et al. (1989) (Table 1).

After 60 mins, all rats were killed and the lenses extracted. The intensity of forward light scattering in the lens was then quantified (Söderberg et al. 1990).

Experiment 2A: anaesthetic, eyelid position and UV-B irradiation versus anterior segment reaction, eyelids sutured

This experiment aimed to compare cataract development after exposure to 300-nm UVR in xylazine/ketamine anaesthetized animals and pentobarbital anaesthetized animals.

Two groups of 20 rats each were treated similarly to those in Experiment 1, with the additional treatment of 5 kJ/m^2 UV-B irradiation at around 300 nm for 15 mins (Fig. 2).

At 30 mins and 60 mins after induction of anaesthesia, the irradiated eyes were inspected with a slit-lamp



Fig. 1. Experiment 1: design. Anaesthetic and eyelid position versus anterior segment reaction; n = 20 in each drug or control group.

 Table 1. Cataract stages (Kufoy et al. 1989).

Stage	Description
0	No cataract
1	Anterior sutural opacity
2	Superficially spread anterior opacity
3	Dense anterior cortical opacity seen without biomicroscope if the eye is laterally illuminated with a pen-light
4	Dense opacity visible even without pen-light



Fig. 2. Experiment 2A: design. Anaesthetic, eyelid position and ultraviolet-B irradiation versus anterior segment reaction; n = 20 in each drug group.

Table 2. Corneal opacity grades (Kufoy et al. 1989).

Stage	Description
0	Clear cornea
1 +	Punctate epithelial keratopathy located in the central portion of the cornea
2 +	Corneal opacity with oedema, anterior chamber structures not seen clearly
3 +	Complete corneal opacity, corneal neovascularization extending from the temporal and nasal bulbar conjunctiva toward the corneal lesion

microscope. The surgical sutures in the irradiated and non-irradiated eyes were released before the rats recovered from anaesthesia. One week after UV-B irradiation, corneal damage was graded according to Kufoy et al. (1989) (Table 2).

Thereafter, the rats were killed. The degree of cataract in the lenses 1 week after UV-B irradiation was quantified *in vitro* as described in Experiment 1.

The 1-week period between UV-B irradiation and cataract assessment was based on our previous finding that cataract development levels out close to an upper asymptote at 1 week after a close-to-threshold 300-nm UVR dose in albino rats (Söderberg 1990a; Michael et al. 1996). The 5kJ/m² dose is slightly above the threshold dose for albino rats anaesthetized with xylazine/ketamine (Michael et al. 1996; Söderberg et al. 2002). It is also the lowest dose to induce permanent cataract in rabbits (Pitts et al. 1977).

Experiment 2B: anaesthetic, eyelid position and UV-B irradiation versus anterior segment reaction, eyelids taped

As in Experiment 2A, this experiment aimed to compare cataract development after exposure to 300-nm UVR in xylazine/ketamine anaesthetized animals and pentobarbital anaesthetized animals, with the addition of enforced proptosis in the pentobarbital group.

The forced lid retraction and proptosis were achieved in the pentobarbital group by circumferential application of adhesive tape. As in Experiment 2A, the xylazine/ketamine group was naturally proptotic. The other eye was kept closed by adhesive tape in both groups (Fig. 3).

Statistics

The sample sizes for experimental groups were based on previously acquired lens light scattering data, accepting an α error of 5% and a β error of 20% in the detection of a 20% difference (Söderberg et al. 1990). All tests were two-sided. Orthogonal *t*-tests were used to test for contrasts. Approximate *t*-tests were used when variances for test groups

were found statistically significantly unequal.

Results

Anaesthesia and eye responses

The xylazine/ketamine anaesthesia was deep, with a total absence of corneal reflex. The pentobarbital group exhibited a weak corneal reflex. The rats anaesthetized with pentobarbital started to recover after about 90 mins, whereas the xylazine/ketamine group remained under anaesthesia for at least 120 mins.

The spontaneously open eyes in the xylazine/ketamine groups (Experiments 1, 2A, 2B) exhibited prominent proptosis. The eyes sutured open in the pentobarbital groups in Experiments 1 and 2A had smaller eyelid openings than eyes in the xylazine/ketamine groups, but the cornea was still exposed to the surrounding air. The eyes taped open in the pentobarbital group in Experiment 2B exhibited prominent proptosis with eyelid retraction, exposing the whole cornea and parts of the sclera. Although there was no difference between xylazine/ketamine groups and pentobarbital groups in the administration of mydriatic eyedrops, the pupil size was slightly smaller in the pentobarbital groups.

Experiment 1: anaesthetics and eyelid position versus anterior segment reaction, eyelids sutured

Corneal surface disturbances and intraocular inflammation

The open eyes in both drug groups exhibited corneal drying at both 30 mins and 60 mins. Corneas in animals that had undergone xylazine/ ketamine anaesthesia tended to show



Fig. 3. Experiment 2B: design. Anaesthetic, eyelid position, forced proptosis and ultraviolet-B irradiation versus anterior segment reaction; n = 20 in each drug group.



Fig. 4. Prevalence of corneal drying in open rat eyes at 30 mins and 60 mins after xylazine/ ketamine (black columns) or pentobarbital (white columns); n = 20 in each drug group. (A) Corneas with < 25% dry area. (B) Corneas with 25-75% dry area. (C) Corneas with >75% dry area.

more corneal drying than corneas in animals that had undergone pentobarbital anaesthesia (Fig. 4).

There were no corneal erosions or signs of anterior chamber inflammation in the non-anaesthetized rats.

Qualitative cataract grading

After anaesthesia with xylazine/ketamine and pentobarbital, respectively, opacities were observed in the superficial anterior lens cortex in the open eyes. Opacities ranged from discrete anterior sutural involvement to dense anterior cataracts. There was a tendency towards a higher prevalence of and more severe cataract after xylazine/ketamine than after pentobarbital anaesthesia (Fig. 5).

After xylazine/ketamine anaesthesia, the cataract seemed to progress towards more severe cataract from 30 mins to 60 mins, whereas after pentobarbital anaesthesia, the cataract seemed to remain constant.

Lenses in non-anaesthetized control animals and lenses in eyes that had been closed during anaesthesia were all clear at examination 60 mins after initiation of anaesthesia.

Intensity of forward light scattering

After 60 mins, the lens light scattering in the xylazine/ketamine open eyes was significantly higher than in the closed eyes (the 95% confidence interval [CI] for mean paired difference excludes 0) (Fig. 6).

There was no difference in lens light scattering between open and closed eyes in the pentobarbital group, nor between the open eyes in the nonanaesthetized control group (95% CIs for mean paired differences include 0).

The difference in lens light scattering between open and closed eyes in the xylazine/ketamine group was significantly larger than in the pentobarbital group (approximate *t*-test for independent groups; test statistic = 7.19, $t_{0.05(2):20} = 2.09$). The 95% CI for mean paired differences between eyes in the pentobarbital and control groups, respectively, included 0 and consequently there was no difference between the two groups (Fig. 6).

Experiment 2A: anaesthetics, eyelid position and UV-B irradiation versus anterior segment reaction, eyelids sutured

Corneal surface disturbances and intraocular reaction

All exposed eyes in both groups exhibited corneal drying 60 mins after induction of anaesthesia, or about 30 mins after UV-B irradiation. Punctate epithelial keratopathy located in the central portion of the cornea was found in half the irradiated eyes in both groups. One week after UV-B irradiation, the prevalence of corneal opacity in irradiated eyes in the xylazine/ketamine group was 85% (Experiment 2A), with hyphaema in 25% of cases. In the pentobarbital group, 25% of the irradiated eyes (Experiment 2A) exhibited corneal opacity (Fig. 7).

Qualitative cataract grading

The incidence of cataract at 60 mins after UV-B irradiation was 90% in the xylazine/ketamine group and 55% in the pentobarbital group. One week later, 55% of the irradiated lenses in the xylazine/ketamine group had anterior cortical cataract. The other 45% could not be inspected due to corneal opacities. In the pentobarbital group, 20% of the lenses exhibited cataract, whereas the other 80% remained clear.

Intensity of forward light scattering

One week after UV-B irradiation, 85% of irradiated lenses in the xylazine/ ketamine group and 20% in the pentobarbital group were cataractous. There were vacuolar opacities in the equatorial region, and irregular opacification in the anterior cortex (Fig. 8A).

No opacities were seen in the nuclear region. All non-irradiated lenses were clear (Fig. 8B).

In rats irradiated under xylazine/ketamine anaesthesia with spontaneous proptosis, the intensity of forward light scattering was higher in irradiated lenses than in non-irradiated contralateral lenses (Fig. 9; 95% CI for the mean paired difference excludes 0).

In rats irradiated under pentobarbital anaesthesia with slight proptosis induced by suture on the upper eyelid and adhesive tape on the lower eyelid, there was no difference in lens light scattering between exposed and contralateral non-exposed lenses (Fig. 9; 95% CI for the mean paired difference includes 0).

The mean paired difference in lens light scattering was higher in the UV-B irradiated xylazine/ketamine group with spontaneous proptosis than in the UV-B irradiated pentobarbital group with slight proptosis induced by suture on the upper eyelid and adhesive tape on the lower eyelid (approximate *t*-test for



Fig. 5. Evolution of cataract stage distribution (0 = no cataract, 4 = dense opacity; Kufoy et al. 1989) in open eyes after (A) xylazine/ketamine or (B) pentobarbital anaesthesia; n = 20 in each drug group.

independent groups; test statistic = 6.10; $t_{0.05(2):21} = 2.08$).

Experiment 2B: anaesthetics, eyelid position and UV-B irradiation versus anterior segment reaction, eyelids taped

Corneal surface disturbances and intraocular reaction

One week after UV-B irradiation, the incidence of corneal opacity in the UV-B irradiated eyes in the xylazine/ketamine group was 90% (Fig. 7; Experiment 2B). Hyphaema was found in 30% of cases. In the pentobarbital group, 70% of irradiated eyes exhibited corneal opacity (Fig. 7; Experiment 2B). The incidence of hyphaema was 15%.

Qualitative cataract grading

At 60 mins after *in vivo* UV-B irradiation under xylazine/ketamine anaesthesia, the prevalence of cataract was 95%. At the same post-exposure interval after *in vivo* UV-B irradiation under pentobarbital anaesthesia with proptosis induced by adhesive tape around the eye, the prevalence of cataract was 70%.

One week after UV-B irradiation under xylazine/ketamine anaesthesia, 55% of the irradiated lenses had anterior cortical cataract, whereas the remaining 45% could not be inspected due to severe corneal opacities and hyphaema. At the same post-exposure interval after *in vivo* UV-B irradiation under pentobarbital anaesthesia with enforced eyelid opening, cataract was found in 55% of eyes, whereas 30% were clear and the remaining 15% could not be inspected due to hyphaema.

Intensity of forward light scattering

One week after UV-B irradiation under xylazine/ketamine anaesthesia with spontaneous proptosis, 90% of irradiated lenses had anterior cortical and equatorial cataract. In the pentobarbital anaesthesia group with proptosis induced by adhesive tape around the eye, 55% of irradiated lenses had anterior cortical and equatorial cataract.

For both treatment groups the intensity of forward light scattering was higher in the irradiated lenses than in the non-irradiated lenses (95% CIs for mean paired differences between irradiated and contralateral non-irradiated lenses exclude 0).

There was no statistically significant difference in lens light scattering between the xylazine/ketamine and the pentobarbital groups, comparing the paired mean differences (approximate *t*-test for independent groups; test statistic = 1.72; $t_{0.05(2):30} = 2.04$) (Fig. 10).

Discussion

This study was designed to quantitatively examine the effect of xylazine/ketamine and pentobarbital anaesthesia, respectively, on lens light scattering. It also aimed to quantitatively study the impact of each of these anaesthetic drugs on *in vivo* UVR-induced cataract development.

In Experiments 1 and 2A, the proptosis occurring during xylazine/ketamine anaesthesia was simulated in the pentobarbital anaesthetized animals by evelid sutures. However, it was noted in these experiments that eyes sutured open did not present the same amount of proptosis as eyes under xvlazine/ketamine anaesthesia. Hence, in Experiment 2B the proptosis in the pentobarbital anaesthetized animals was increased by using adhesive tape around the eye. This increased the proptosis, but the eye opening was still less than that observed under xylazine/ketamine anaesthesia.

That there was a higher prevalence of and more widespread surface



Fig. 6. Intensity of lens light scattering after xylazine/ketamine (spontaneous proptosis) or pentobarbital (slight proptosis by suture in the upper lid and adhesive tape on the lower lid) versus non-anaesthetized control. Closed eyes (black columns) or pentobarbital (white columns) and mean paired difference (shaded columns). Right (R) and left (L) lenses for the non-anaesthetized control group are shown. Bars represent 95% confidence intervals for the mean; n = 20.



Fig. 7. Distribution of corneal opacity 1 week after 5 kJ/m² ultraviolet-B irradiation in rats anaesthetized with xylazine/ketamine or pentobarbital; n = 20 in each group. Grading according to Kufoy et al. (1989).



Fig. 8. Macroscopic appearance of (A) the rat lens 1 week after 5 kJ/m² ultraviolet-B irradiation under xylazine/ketamine anaesthesia and (B) the non-irradiated contralateral lens. Mesh size = 0.79 mm.

corneal drying at 30 mins and 60 mins in the open eyes in the non-irradiated xylazine/ketamine group than in the non-irradiated pentobarbital group is consistent with previous data (Calderone et al. 1986) and conforms with the evaporation-dehydration hypothesis proposed by Fraunfelder & Burns (1970). This might be due to a specific drug-induced effect in the cornea. However, the fact that we noticed greater proptosis in animals anaesthetized with xylazine/ketamine than in animals anaesthetized with pentobarbital with eyelids sutured open suggests that corneal drying is related to the degree of proptosis and eyelid retraction.

The finding in Experiments 2A and 2B that corneal opacification after UV-B irradiation became almost as pronounced in animals under pentobarbital anaesthesia with pronounced proptosis induced by adhesive tape as it did in animals under xylazine/ketamine anaesthesia with spontaneous proptosis indicates that it is the proptosis rather than the drug that determines the corneal opacification.

The absence of cataract in all closed eyes at 60 mins and 1 week after anaesthesia serves as proof against a primary drug-induced cataractogenic effect. The observed reversible cataract in proptotic eyes during anaesthesia without UV-B irradiation has primarily been related to dehydration. In addition, low-temperature or cold cataract has also been suggested as a possible cause of reversible cataract (Fraunfelder & Burns 1970). Classic cold cataract is caused by the aggregation of proteins due to phase shifting. The lens nucleus has the highest level of protein concentration and is therefore the normal location for cold cataract. Cold cataract can develop at temperatures as high as 26 ° (Mizuno 1984). Although xylazine/ketamine induces hypothermia (Erhardt et al. 1984; Livingston et al. 1984), the body temperature does not decrease to the levels at which cold cataract usually occurs (Fraunfelder & Burns 1970). The combination of lowered body temperature and increased loss of heat from the eye by increased evaporation from the cornea and loss of heating from the eyelids may play a role in transient cataract development, although not as classic cataract with phase shifting, but instead by disturbing ion pumps, and subsequently disturbing the osmotic balance. Until a direct determination of temperature in the anterior lens cortex has been undertaken, the purist should not entirely reject low temperature as a contributing factor in transient anaesthesiainduced cataract.

In the first UVR experiment (2A) the irradiated lenses in the



Fig. 9. Intensity of lens forward light scattering 1 week after ultraviolet-B irradiation under anaesthesia with xylazine/ketamine (spontaneous proptosis) or pentobarbital (proptosis induced by a suture in the upper lid and adhesive tape on the lower lid). White columns represent irradiated eyes (proptosis during irradiation). Black columns represent non-irradiated eyes (closed during irradiation). Shaded columns represent the mean paired difference. Bars represent 95% confidence intervals for the mean; n = 20.



Fig. 10. Intensity of lens light scattering 1 week after *in vivo* ultraviolet-B irradiation in rats under anaesthesia with xylazine/ketamine (spontaneous proptosis) or pentobarbital (proptosis induced by adhesive tape around the eye). White columns represent irradiated eyes (proptosis during irradiation). Black columns represent non-irradiated eyes (closed during irradiation). Shaded columns represent the mean paired difference. Bars represent 95% confidence intervals for the mean; n = 20.

xylazine/ketamine group developed significant cataract after 1 week, whereas the majority of lenses in the pentobarbital group, receiving the same dose, remained clear. This is probably explained by the larger irradiated area of the proptotic eyes in animals under xylazine/ketamine anaesthesia. The larger area of cornea and sclera exposed to air and UVR in xylazine/ketamine group in the Experiments 2A and 2B also explains the increased corneal damage, at both 1 hour and 1 week after irradiation. Because a larger area of cornea and sclera was exposed to UV-B irradiation in the xylazine/ketamine rats, their eyes and lenses received a higher dose of UVR-B than did those of the pentobarbital rats. The higher incidence of hyphaema in irradiated eyes in the xylazine/ketamine groups also

supports this. This conclusion is further supported by the outcome of UV-B irradiation in eyes with pronounced proptosis induced by adhesive tape around the eye in pentobarbital-anaesthetized animals. The incidences of hyphaema and cataract approached that in the xylazine/ketamine group. Optical theory leads us to expect this as a larger eyelid opening provides a larger solid angle for acceptance of UVR energy into the eye.

In the second UVR experiment (2B), the difference in lens light scattering between the two anaesthesia groups became smaller and statistically non-significant. This is explained by the more extreme proptosis and eyelid retraction achieved in the pentobarbital group by the circular application of adhesive tape. There have been speculations about photosensitive effects in the development of anaesthesia-related cataract, although Fraunfelder & Burns (1962) and Dietze et al. (1974) rejected this theory. Our data do not warrant a complete rejection of a UVR photoprotective effect of pentobarbital or a photosensitizing effect of xylazine/ketamine, but the most plausible explanation is that more extreme proptosis was achieved with xylazine/ketamine anaesthesia than with pentobarbital anaesthesia, despite the proptosis induced by adhesive taping in the pentobarbital group.

The current study demonstrates that the choice of anaesthetic strongly influences the outcome of ophthalmic experiments. We have demonstrated that xylazine/ketamine anaesthesia facilitates the development of UVRinduced cataract compared with pentobarbital anaesthesia. The main influencing factor seems to be the degree to which the eyelid covers the eye. Our data indicate that it is preferable to use xylazine/ketamine anaesthesia in order to optimize the signal : noise ratio in experiments aiming at determining risk of UVR exposure to the eye. Further, eyelid opening must be considered when relating data to exposure in day-today life.

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