Heliyon 6 (2020) e05686

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Intravenous ketamine for long term anesthesia in rats

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ARTICLE INFO

Keywords:
Biological sciences
Neuroscience
Physiology
Ophthalmology
Laboratory medicine
Rat
Anesthesia
Ketamine
Xylazine
Electroretinogram
ERG
Retina

ABSTRACT

Ketamine/xylazine anesthesia has been used primarily for short term procedures in animals, but two prior reports used intravenous ketamine/xylazine for experiments taking many hours. However, there is a discrepancy about the appropriate dose, which is resolved here. Adult Long-Evans rats were used for recording from the retina. Doses of Ketamine/xylazine were adjusted to minimize anesthetic in terminal experiments lasting 10 h. An allometric relation was fitted to the resulting data on doses as a function of body weight, and compared to prior work. The allometric relationship between the continuously infused specific dose and weight was: dose = 9.13 (weight)$^{-1.213}$ ($r^2 = 0.73$), where dose is in mg-kg^{-1}-hr^{-1} and rat weight is in kg. The dose of xylazine was 3.3% of the ketamine dose. No attempt was made to explore different relative doses of xylazine and ketamine. Prior work is consistent with this relationship, showing that the earlier discrepancy resulted from using rats of different sizes. Ketamine at the doses used here still depressed the electroretinogram relative to historical controls using urethane. We conclude that intravenous ketamine dosing in rats should not use the same mg-kg^{-1}-hr^{-1} dose for all rats, but take into account the strong allometric relationship between dose and rat weight. There is an advantage in using smaller doses in order to prevent unnecessary depression of neural responses.

1. Introduction

While ketamine anesthesia is most often used for relatively brief procedures in animals, there are three reports explaining how it can be used for many hours in rats (Barriga-Rivera et al., 2018; Simpson, 1997; Ritschl et al., 2015). Simpson (Simpson, 1997), working with Sprague-Dawley rats, obtained stable anesthesia for 12 h with intravenous (IV) ketamine at 60-75 mg-kg^{-1}-hr^{-1} and xylazine at 1.9–2.4 mg-kg^{-1}-hr^{-1}. Barriga-Rivera et al. (Barriga-Rivera et al., 2018) used Long-Evans rats, and achieved anesthesia with about half the dose used by Simpson, 24.0–34.5 mg-kg^{-1}-hr^{-1} with 0.9–1.2 mg-kg^{-1}-hr^{-1} of xylazine in recordings over 15 h. Barriga-Rivera et al. supplemented the IV anesthesia with a small amount of isoflurane (0–0.5%). The third report of intravenous ketamine (Ritschl et al., 2015) did not use a continuous infusion, but supported the value of IV ketamine over intraperitoneal ketamine for experiments lasting even longer. Encouraged by the earliest of these reports (the second was not available when we began), we have also used ketamine combined with xylazine for more than ten hours in terminal experiments on rats in which recordings were made from the retina. Our rationale for using ketamine was that for a particular set of experiments, we wanted to use the same anesthetic for short-term recovery experiments and for terminal experiments on the same animals, which ruled out the very long-acting agent urethane, which is also hypotensive if given rapidly. Isoflurane, which might have been suitable otherwise, was excluded because it is a vasodilator and increases retinal blood flow (Li et al., 2008; Moult et al., 2017; Muir and Duong, 2011), which was an important measurement in our experiments. The purposes of this report are to show that the different doses used by Simpson (Simpson, 1997) and Barriga-Rivera et al. (Barriga-Rivera et al., 2018) are not in conflict if one takes into account an allometric relation for rats of different weights, and to document the influence of ketamine, in comparison to urethane, on the electroretinogram.

2. Methods

Experiments were performed on 17 adult male Long-Evans rats purchased from Envigo (www.envigo.com). They weighed 333 ± 52 g (232–432 g), and were between 8 and 14 weeks old at the time of experiments. The animal use followed the NIH Guide for the Care and Use of Laboratory Animals and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by Northwestern University's

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https://doi.org/10.1016/j.heliyon.2020.e05686
Received 26 August 2020; Received in revised form 15 October 2020; Accepted 4 December 2020

Institutional Animal Care and Use Committee. Animals were housed conventionally in the animal care facility with 12h-12h light dark cycles, access to enrichment, and standard diets. For experiments, rats were anesthetized with 3% isoflurane/35% O_2 and given an intramuscular dose of 37 mg/kg ketamine (Ketathesia, 100 mg/ml) and 7 mg/kg xylazine (AnaSed, 20 mg/ml). Isoflurane was turned off, and after the ketamine took effect, as judged by the lack of response to toe pinch, a rectal probe was inserted to monitor temperature and the rat was placed on a heating pad with circulating 38 °C water, and an infrared lamp powered by a variable transformer was placed above the rat to assist with temperature control. A pulse oximeter (Nonin, Plymouth, MN) was placed on a foot to measure heart rate and arterial oxygen saturation (SpO_2), and inspired air was supplemented with 100% O_2 as necessary to maintain SpO_2 above 90%. Surgery was performed to insert a tracheotomy tube (12 gauge stainless steel), and cannulas (PE 50, Becton-Dickinson) into both femoral veins (for independent control of anesthesia and paralytic) and one femoral artery (for blood pressure and arterial samples). Eye surgery was done as described previously (Lau and Linsenmeier, 2012). This allowed the eye to be attached to a plate sewn to the sclera that stabilized the eye and permitted penetration with a needle to carry a double-barreled microelectrode for intraretinal recording of PO_2 and vitreal or intraretinal recording of the electroretinogram (ERG) (Lau and Linsenmeier, 2012; Linsenmeier and Yancey, 1987). Frequently an additional IM bolus of ketamine/xylazine, usually half the initial dose, was needed during early phases of surgery if the response to pinch showed that the anesthesia was becoming too light. A prophylactic dose of 20 mg/kg of cefazolin was given IV to limit infection.

About one hour after the beginning of surgery (which took more than two hours), when pinch tests showed that the animal needed more anesthetic, IV delivery was started with a mixture of 0.6 ml of 100 mg/ml ketamine, 0.1 ml of 20 mg/ml xylazine and 6 ml saline at a pump rate of 1.6 ± 0.5 ml/h (an average of 14.2 mg/h of ketamine and 0.47 mg/h of xylazine). Because ketamine and xylazine were always mixed in the same proportion, only the ketamine doses are given below. The IV xylazine dose in mg/hr was always 3.3% of the ketamine dose as used in previous studies. Sometimes the initial rate of infusion was too little, based on responses to pinch, and bolus IV infusions of 0.2 ml were needed until the IV infusion took hold and the correct rate of infusion was found. As seen in Figure 1, adjustments were made as needed. After surgery was complete and the IV infusion started, the rat was transferred to a Faraday cage. The rectal probe was connected to a feedback controlled water bath that supplied a water blanket around the animal. The arterial cannula was connected to a Harvard Apparatus (Holliston, MA) transducer to monitor blood pressure. EKG leads were inserted into the forelimbs and connected to an amplifier and an oscilloscope, and fed to a second amplifier that generated a pulse played through a loudspeaker during the rest of the preparation and recording period, allowing auditory monitoring of the heart rate. Xylocaine gel was instilled into the ears and ear bars were inserted to hold the head. The eye was attached to the stabilizing plate. After these preparations were completed, and after no adjustments in the ketamine/xylazine infusion rate had been made for at least 45 min while checking stability of heart rate and breathing, and ensuring the absence of palpebral and pinch responses, a bolus infusion of 0.3 mg of Pavulon (pancuronium bromide) was given and the animal was connected to a respirator set at 60 breaths/min. Paralysis was done to provide further eye stability during intraretinal recording, and to ensure that the arterial PO_2, PCO_2 and pH (P_aO_2, P_aCO_2 and pH_a) could be controlled by adjustments of the tidal volume and the inspired gas. Pavulon was continued at 0.5 mg-kg^{-1}-hr^{-1} and ketamine/xylazine was also continued via a separate venous cannula during the recording period, which lasted until euthanasia with IV saturated KCl. The oxygen-sensitive double-barreled microelectrode was introduced into the eye, and many penetrations were done to measure retinal PO_2 and the vitreal or intraretinal ERG in response to flashes of light, as described previously (Lau and Linsenmeier, 2012). Animals were dark-adapted during these recordings, and the retina recovered quickly from the 2.5 s flashes of diffuse white light used to elicit the ERGs. No potentially painful manipulations were done following paralysis. The time from starting IV ketamine/xylazine to euthanasia was 10 ± 1.5 h (mean and SD). Arterial samples of 0.2 ml were taken approximately hourly and P_aO_2, P_aCO_2 and pH_a were measured with a blood gas analyzer (Siemens 248, Siemens Medical Solutions, Malvern, PA).

The procedures described above were used for 12 of the 17 animals. In those animals, optical coherence tomography (OCT) imaging of the same eye with visible light (Yi et al., 2015) had been done on the preceding day, using induction with isoflurane and IM ketamine, a procedure that took about 1.5 h. There was no surgery and no IV ketamine on the day of imaging. The animals were allowed to recover from anesthesia and were returned to animal housing before performing the procedures described above for the terminal experiment. For the first 5 animals, however, we attempted to do both imaging and microelectrode recording on the same day. In those cases we completed most of the surgery described above, including initiation of IV ketamine/xylazine, performed the imaging, and then performed the intraretinal recording. The interposition of the imaging procedure in those cases did not affect the doses of IV ketamine that were used, so results from all animals are presented here. We changed from the one day procedure to the two day procedure because the ERG amplitudes in the one day procedure were smaller than expected, and we suspected that inadequate recovery from the light used for imaging was the cause. In the two day procedure the animal had time to recover after the strong imaging light before the intraretinal recordings were done.

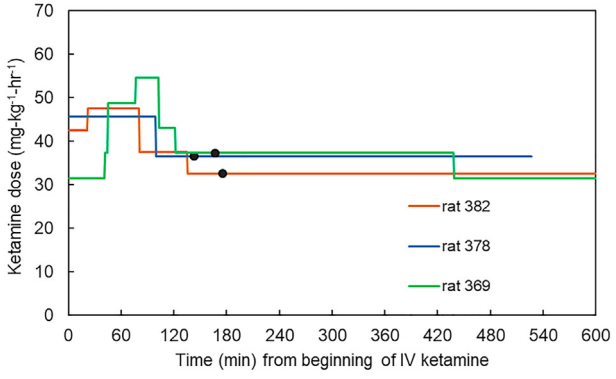

Figure 1. Dose of ketamine (mg-kg^{-1}-hr^{-1}) administered intravenously to three rats as a function of time. Xylazine was mixed with ketamine and administered at 3.3% of the dose rate of ketamine in all cases. Filled circles show when the animal was paralyzed with pancuronium bromide and artificially respirated.

3. Results

Figure 1 shows examples of the way in which the dose rate was adjusted in three animals. Zero time is the beginning of the IV delivery of ketamine/xylazine. Doses were adjusted as surgery continued to a final value that was generally maintained until euthanasia, although in one of these cases, and in others, falling blood pressure and heart rate led to a small decrease in ketamine dose rate later. The final value was an attempt to minimize the dose of anesthetic consistent with the maintenance of anesthesia sufficient to keep heart rate and breathing steady and eliminate palpebral and pinch reflexes. The mean blood pressure, recorded at the time of blood gas measurements, for the 17 animals, was 92.1 ± 14.2 mm Hg (mean and SD), and the heart rate was 258.7 ± 29.3 min^{-1}.

Black circles in Figure 2a show, for each animal in the present study, the time-averaged doses ([$\sum$(rate$_i$ x time$_i$)/total time], where i is an episode of anesthesia during time$_i$ at rate$_i$) over the time of IV infusion. Figure 2b shows the final running dose (i.e. after all adjustments and until euthanasia) for each animal. Also shown on these plots are values

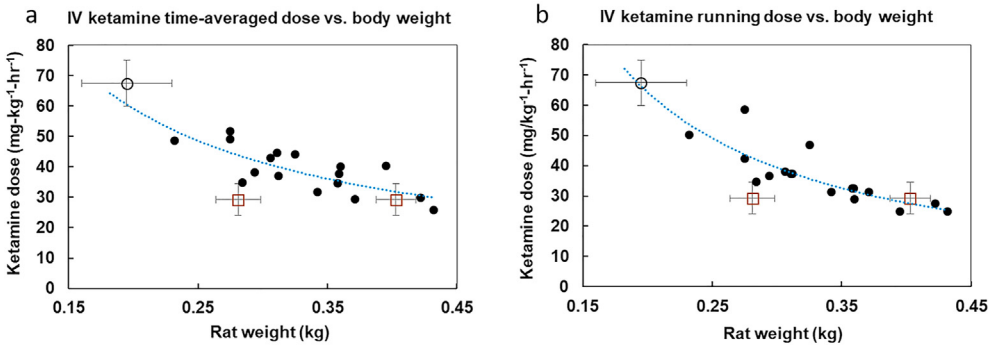

Figure 2. Ketamine doses as a function of body weight. a. Time-averaged specific ketamine dose rate (mg-kg^{-1}-hr^{-1}) for each animal (filled circles). The average dose from (Simpson, 1997) shown as an open circle, and average doses from (Barriga-Rivera et al., 2018) are shown as open squares. The square at the lower body weight is for females and the one at the higher body weight is for males. The error bars for the open circle and squares are ranges rather than standard deviations, which were not available in those references, except for the horizontal error bars for the open squares, which show the standard deviation. The regression line is a power law fit: dose = 14.27 (weight)$^{-0.884}$ (r^2 = 0.56; p < 0.001) where dose is in mg-kg^{-1}-hr^{-1} and weight is in kg. b. Final running specific dose rate for each animal (filled circles). As in a, filled circles each represent one animal in this study, and open symbols are from previous studies. The regression line is a power law fit: dose = 9.13 (weight)$^{-1.213}$ (r^2 = 0.73; p<<0.001).

from the previous studies. Males and females were reported separately by Barriga-Rivera et al.(Barriga-Rivera et al., 2018). The animals in Simpson's study (Simpson, 1997) and in ours were all males. It is not clear whether the earlier studies reported final anesthetic dose rates or time-averaged rates, so the same values from those studies are used for both graphs. Only the black points are fitted with power laws that are given in the legend. Note that smaller animals required higher specific doses; that is, the dose per kg of body weight obeyed an allometric relationship. In part b of the Figure 2, this is dose = 9.13 (weight)$^{-1.213}$ (r^2 = 0.73), where weight is in kg and dose is in mg-kg^{-1}-hr^{-1}. Simply using a single rate of administration in mg-kg^{-1}-hr^{-1} and finding the mg/hr dose based on each animal's weight would have led to doses that were unnecessarily high for larger animals. No assumption about an allometric relationship was made during the experiments. We adjusted the dose in each animal as needed, and only after most of the experiments were completed did we discover this relationship. Linear fits to the data from the present study would also have led to acceptable fits, but when extrapolated, the regression line would have fallen below Simpson's data, and a power law is more in line with expectations. The data from Barriga-Rivera et al., especially for males, also falls on the regression lines.

One additional study (Ritschl et al., 2015) used IV ketamine in male Wistar rats, without xylazine after the induction dose, for nearly 22 h. Ketamine was given in bolus doses as needed rather than continuously. From the total duration, average total volume of infusion, and ketamine concentration that they provide, and assuming an average rat weight of 325 g from their statement that rats weighed between 300 and 350 g, one can derive an equivalent infusion rate of 40 mg-kg^{-1}-hr^{-1}. This value is not plotted in Figure 2, because of the difference in method of administration and assumptions involved, but this calculated value falls exactly on the regression line in Figure 2a.

We also analyzed the effect of ketamine on the electroretinogram recorded with the microelectrode in the vitreous humor, referenced to an Ag/AgCl electrode beneath the skin on the neck or back. As noted in methods, the ERGs were small when OCT imaging was performed on the same day as ERG measurements, so only the ERGs recorded in the 12 terminal experiments performed on a different day than imaging are reported. Figure 3 shows an intensity-response series of ERGs from one animal in response to 2.5 s diffuse flashes of white light. They are characterized by a small maintained response (called the d.c. component (Brown, 1968)) at weak illuminations. At higher illuminations there is a transient positive-going potential (b-wave) followed by a slower potential (the-c-wave) that is positive-going at intermediate illumination, but tends to be negative-going in rats at higher illumination. Flashes were not bright enough to evoke a-waves.

Figure 4 shows how the b- and c-wave amplitudes varied with illumination in the ketamine experiments, compared to the ERGs recorded in a set of historic control animals anesthetized with urethane, but otherwise prepared in the same way, from previous work

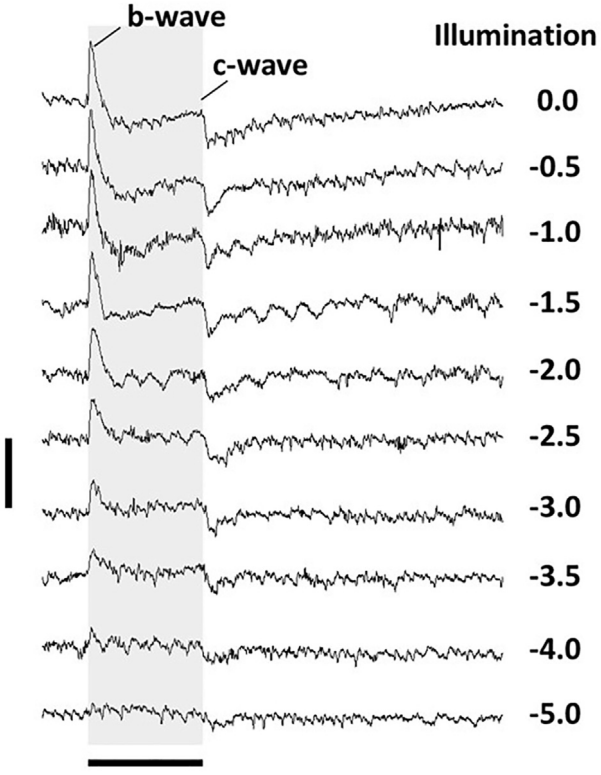

Figure 3. The electroretinogram at different illuminations recorded in one animal. The stimulus illumination is given in terms of log units relative to the maximum available, which was about 2 log units above rod saturation. The gray bar and black line below the traces indicate the stimulus duration of 2.5 s, and the vertical line is 0.5 mV.

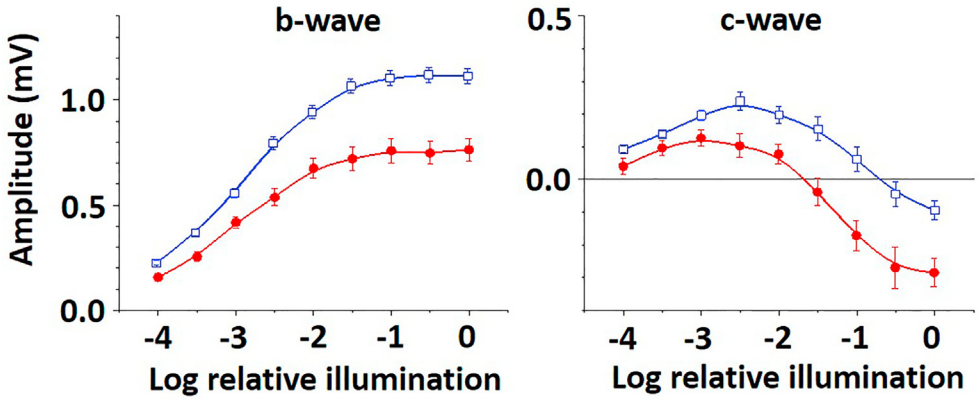

Figure 4. Average intensity response curves for the b-wave and c-wave from stimulus-response functions like that shown in Figure 3 from urethane-anesthetized animals (blue) (n = 40 from 27 animals) and ketamine-anesthetized animals (red) (n = 17 from 12 animals). Points are mean and standard error of the mean.

(Dmitriev et al., 2016a, 2016b, 2019). The ERGs from ketamine-anesthetized animals were markedly smaller than those from urethane-anesthetized animals. Because the OCT imaging had reduced the ERGs when imaging and electrophysiology were done on the same day, it seemed possible that there was a residual effect of imaging even a day later, when the recordings in Figure 4 were made. This cannot be entirely ruled out, but in one animal, toward the end of the recording period, we increased the ketamine dose and tracked the ERG, as shown in Figure 5. Within 10 min of increasing the ketamine dose, at a time when only 3.7 mg more ketamine had been infused, the b-wave had been reduced to 75% of its initial amplitude, and after 20 min it had been reduced to 50%, implicating ketamine as being responsible for much if not all of the reduced ERG in ketamine-anesthetized animals.

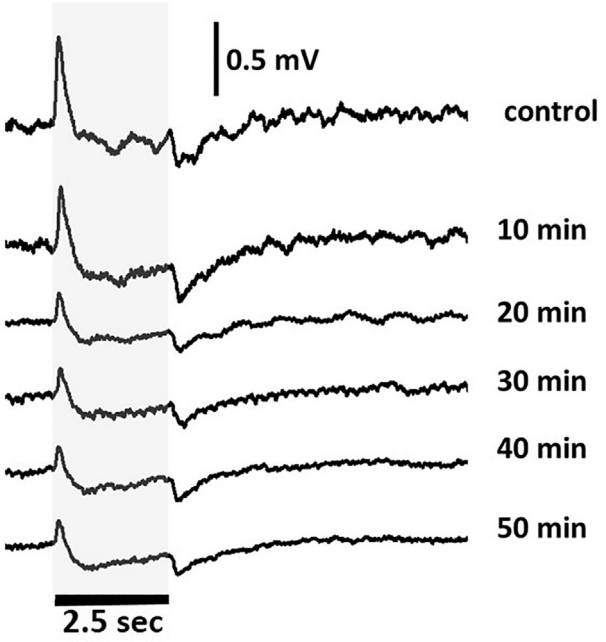

Figure 5. Depression of the electroretinogram (ERG) by increased ketamine. Each response is an average of 5 individual vitreal ERG responses to -1.0 relative log illumination obtained with 2.5 s flashes. The rate of ketamine administration was 10.7 mg/h for several hours preceding and during the control response. At time = 0, the rate was increased to 22.4 mg/h, and responses at times after this are indicated.

4. Discussion

We found, in agreement with previous work, that ketamine can be useful when given IV for long term experiments in rats. As Simpson found, adjustments in ketamine dose rate were needed for each rat initially to establish an appropriate dose. Here we found that the specific dose rate was not constant, but needed to be adjusted according to a power law, and that this brought the higher doses that Simpson used, and the lower doses of Barriga-Rivera et al., into alignment with ours. A lower dose per kg was needed in larger animals. Simpson's rate of infusion now appears to have been higher because the animals in that study were smaller. It is possible that sex or age (Veilleux-Lemieux et al., 2013) may play an additional role, but this cannot be determined with certainty from the data available.

Allometric effects with power laws similar to the one found here are observed in analyzing a variety of functions that are ultimately tied to metabolism. Large animals have higher *total* metabolic rates, but when expressed as *specific* metabolic rates, per kg of body weight, the exponents of the power laws are negative and usually between -0.5 and -1. Thus, one cannot use a single dose (per kg) of a drug for all body weights, nor can one usually scale up or down linearly (i.e. with an exponent of -1 or 1). Allometric relationships provide a good description of physiological functions ranging from the well-known result that small animals have much higher specific oxidative metabolic rates (Kleiber, 1961; Schmidt-Nielsen, 1984; White and Seymour, 2005), to the higher heart rates and breathing rates of smaller animals (Seymour and Blaylock, 2000; Stahl, 1967), to differences in drug metabolism between infant and adult humans (Knibbe et al., 2005). There are principles for finding allometric relations for drug doses across species (Sharma and McNeill, 2009). Riviere et al. (Riviere et al., 1997) attempted to generate an allometric relationship for ketamine across five species, the smallest of which was cat. It was expected that because ketamine is eliminated in a flow-limited way by the kidney, and glomerular filtration rate is known to be allometric, this should have been possible. However, the intraspecific regression for the power law relating half-life to weight among those species was not significant. Nevertheless, because of the elimination mechanism for ketamine, it might be expected that an *intra*specific regression would be successful, as found here. We would not try to apply the relationship found here to other species, but the data show that the required doses for three strains of rats (Long-Evans, Sprague Dawley, and Wistar) are the same. However, it was somewhat surprising that the specific ketamine dose would vary substantially within the range of rat body weight with a relatively large exponent of the power law. It should be noted that the power law we found was based on effects of the drug, in contrast to pharmacokinetic data, which might give a different exponent.

It is also worth noting that even though the power law gives a good fit, there may be individual differences in rats, so they all still have to be monitored carefully.

As noted earlier, we did not try to adjust the relative dose of ketamine and xylazine, but reducing xylazine over time might be worth exploring, because it may have negative impacts on respiration and blood pressure.

Ritschl et al. (Ritschl et al., 2015) used a more rapid method of cannulation, with microcatheters designed for newborns (1 French = 1/3 mm), either in the femoral vein or in the jugular vein. This would be more suitable for experiments in which the animals recover from anesthesia. Significantly, Rischl et al. (2015) had more success in prolonged experiments with intravenous injections than with repeated peritoneal injections.

Anesthetics are designed to depress parts of the nervous system responsible for pain and consciousness, but their effects are not restricted to those functions, and there is a long history of analyzing anesthetic effects on the ERG (Jeong et al., 2009; Millar et al., 1989; Nair et al., 2011; Vaegan et al., 1990; van Norren and Padmos, 1977; Woodward et al., 2007), which is technically easier than measuring other aspects of retinal function. Nair et al. (Nair et al., 2011) reported that ketamine/xylazine anesthesia allowed larger ERG responses in Sprague-Dawley rats than urethane, the opposite of our experience (Figure 4). The reason for this difference is not certain. It may be the duration of the experiments, which was undoubtedly shorter in the experiments by Nair et al. (Nair et al., 2011), so that ketamine had less cumulative effect, or could be related to their single large dose (1 g/kg IP) of urethane. In our previous work we gave a loading dose of urethane of 0.8 g/kg IV over two or three hours, which gradually replaced isoflurane, and then gave 75 mg-kg^{-1}-hr^{-1} continuously. There may also be a strain difference in ERG sensitivity to anesthetics, but it was clear that ketamine was responsible for depression of the ERG in our experiments. The mechanism of this effect is not known, but it cannot be due to the influence of ketamine on NMDA receptors, because the b- and c-waves of the ERG are generated by responses of bipolar cells, retinal pigment epithelial cells, and Muller glial cells, which occur distally in the retina, and are not influenced by the NMDA receptors, which are only in proximal retina (Jakobs et al., 2007). In cats, Vaegan et al. (Vaegan et al., 1990) reported that urethane allowed pattern ERG responses that were similar to those in decerebrate cats, while ketamine/xylazine altered pattern responses, depressing them at low spatial frequencies and enhancing them at high spatial frequencies. While ketamine depresses some functions, one advantage of ketamine is that, in contrast to inhalational anesthetics, it did not prolong dark adaptation times in primates (van Norren and Padmos, 1977).

We conclude that ketamine/xylazine can be a good anesthetic for long term experiments in rats, and that the dose for rats of different sizes can be predicted from an allometric relation. However, because ketamine at the doses used here led to decreased visual responses relative to those observed with long term urethane anesthesia, it would be prudent to compare physiological responses under ketamine and under an alternate anesthetic before deciding which is best for a particular set of experiments.

Declarations

Author contribution statement

R. Linsenmeier: 1, 2, 3, 5; Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

L. Beckmann: Conceived and designed the experiments; Performed the experiments.

A.V. Dmitriev: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by the National Institutes of Health (R21EY027502).

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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