Aged mice show an increased mortality after anesthesia with a standard dose of ketamine/xylazine

Sandra Schuetze1,2*, Anja Manig1,3, Sandra Ribes1 and Roland Nau1,4

Abstract
Geriatric animal models are crucial for a better understanding and an improved therapy of age-related diseases. We observed a high mortality of aged mice after anesthesia with a standard dose of ketamine/xylazine, an anesthetic regimen frequently used in laboratory veterinary medicine. C57BL/6-N mice at the age of 2.14 ± 0.23 months (young mice) and 26.31 ± 2.15 months (aged mice) were anesthetized by intraperitoneal injection of 2 mg ketamine and 0.2 mg xylazine. 4 of 26 aged mice (15.4%) but none of 26 young mice died within 15 min after injection of the anesthetics. The weight of aged mice was significantly higher than that of young mice (32.8 ± 5.4 g versus 23.2 ± 3.4 g, p < 0.0001). Thus, aged mice received lower doses of anesthetics in relation to their body weight which are within the lower range of doses recommended in the literature or even beneath. There were no differences between deceased and surviving aged mice concerning their sex, weight and their motor performance prior to anesthesia.

Our data clearly show an age-related increase of mortality upon anesthesia with low standard doses of ketamine/xylazine. Assessment of weight and motor performance did not help to predict vulnerability of aged mice to the anesthetics. Caution is necessary when this common anesthetic regimen is applied in aged mice: lower doses or the use of alternative anesthetics should be considered to avoid unexpected mortality. The present data from our geriatric mouse model strongly corroborate an age-adjusted reduction of anesthetic doses to reduce anesthesia-related mortality in aged individuals.

Keywords: Aging, Anesthetics, Geriatric mouse model, Mortality, C57BL/6

Introduction
The increased life expectancy is going hand in hand with an increase of age-related diseases, such as neurodegenerative diseases, cancer, and atherosclerosis [1]. Aged persons are more vulnerable to infections and other external stressors [2, 3]. Because of the age-related decline of organ functions and age-related changes of pharmacokinetic and pharmacodynamic features, elderly individuals react differently to therapeutics and anesthetics [4, 5]. To improve therapies for the growing group of geriatric persons, age-related diseases and conditions require proper scientific investigation. In clinical studies, nowadays there is a trend to pay more attention to persons at an age over 80 years, which have been excluded from many studies in the past [6, 7]. Geriatric animal models are needed for a better understanding of age-related changes and processes. Recently, geriatric mouse models for frailty and sarcopenia including C57BL/6 mice up to an age of 28 months have been established [8–10]. Thus, aged mice become increasingly important for basic research. This is reflected by the fact that animal breeding companies started to offer aged mice up to an age of more than 24 months. Many interventions during mouse experiments require anesthesia, and the ketamine/xylazine regimen is widely used [11–14]. There are some studies which primarily focus on the effects of these anesthetics on the mouse creature [15–18], the influence of mouse strain and sex on the susceptibility to anesthetics [19], or the optimization...
of anesthesia protocols in mice [20–22]. Although advanced age over 18 months has been considered to influence susceptibility of mice to anesthetics [13], to our knowledge, experimental studies or recommendations primarily addressing anesthetic regimes for geriatric mice do not exist so far. ARRIVE guidelines specify to pay attention to anesthesia [23]. However, the crucial role of anesthetics is often ignored or underestimated in the experimental design and later publication of animal research models [24, 25].

We established a geriatric mouse model for Escherichia coli (E. coli) meningitis [26], in which young and aged mice received intraperitoneal anesthesia with standard doses of ketamine/xylazine before intracerebral injection of E. coli K1. During these experiments, we observed a substantial mortality of aged mice under this standard anesthetic regimen frequently used in laboratory veterinary medicine. We consider this observation highly relevant for our further work with geriatric mouse models and for other researchers performing experiments with aged mice.

Methods/ experimental Animals
Animal experiments were approved by the Animal Care Committee of the University Medical Center Göttingen, Germany, and by the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Braunschweig, Lower Saxony, Germany.

C57BL/6-N mice were bred in the animal care facility (Zentrale Tierexperimentelle Einrichtung) of the of the University Medical Center Göttingen, Germany, and housed under a 12:12 h light:dark cyle, 20°C room temperature and 55% humidity in compatible groups of maximum 5 animals until they reached the intended age. They were provided with free choice standard rodent diet and bottled tap water. 26 C57BL/6-N mice at the age of 2.14 ± 0.23 months (young mice) and 26 C57BL/6-N mice at the age of 26.31 ± 2.15 months (aged mice) received anesthesia. Prior to anesthesia, all mice were manually restrained, and 30 μl of the anesthetic mixture were injected intraperitoneally into the right lower quadrant of the abdomen using a 25-gauge needle. Independently of the body weight, each mouse received 2 mg ketamine and 0.2 mg xylazine.

Statistics
GraphPad Prism 5.0 Software (GraphPad Software, San Diego, California, USA) was used to perform statistical analyses and graphical presentation. Log-rank test was performed for the comparison of survival curves. Weights of mice and doses of anesthetics are expressed as means±standard deviations (SD) and were compared using the Student’s t-test. The tight rope test scores are expressed as medians (25th percentile/75th percentile). Mann-Whitney U-test was performed to compare the tight rope test scores between the groups. Sex distributions were compared using the Chi-squared test. P-values < 0.05 were considered statistically significant.

Results
4 of 26 aged mice (15.4%) died within 15 min after injection of ketamine/xylazine (2 mg/0.2 mg), whereas none of the 26 young mice died from the anesthesia (p = 0.039; Fig. 1).

Sex distribution was similar in both groups [young mice: 17 of 26 mice female (65%), aged mice: 18 of 26 mice female (69%); Table 1]. Weight of aged mice (32.8 ± 5.4 g) was substantially higher than that of young mice (23.2 ± 3.4 g; p < 0.0001; Fig. 2a, Table 1). Young mice showed a better motor performance than aged mice as assessed by the tight rope test, with lower scores indicating a better performance: tight rope test scores [medians (25th percentile/75th percentile)] of young mice were significantly lower than those of aged mice [1(1/2) versus 16(9.75/18.25); p < 0.0001; Fig. 2b]. There was no significant difference between surviving and deceased aged mice concerning their body weight (33.3 ± 5.0 g versus 30.2 ± 7.5 g; p = 0.30; Fig. 2a, Table 1) and their tight rope test scores [16(10/18.25) versus 11(2.5/19.5); p = 0.83; Fig. 2b].

Ages of surviving and deceased aged mice did not differ significantly (p = 0.49; Table 1), and sex distribution of surviving and deceased aged mice was similar (p = 0.79; Table 1).

Anesthetic dilution and administration
Ketamine 10% (100 mg/ml; Medistar, Ascheberg, Germany) and xylazine 2% (20 mg/ml; Riemser, Greifswald, Germany) were combined in a single insulin syringe (2 parts of ketamine and 1 part of xylazine). Mice were manually restrained, and 30 μl of the anesthetic mixture were injected intraperitoneally into the right lower quadrant of the abdomen using a 25-gauge needle.
In relation to their body weight, aged mice received approximately 70% of the doses of ketamine and xylazine administered to young mice (aged mice: 62.6 ± 10.3 mg/kg ketamine, 6.3 ± 1.0 mg/kg xylazine; young mice: 87.9 ± 12.0 mg/kg ketamine, 8.8 ± 1.2 mg/kg xylazine; p < 0.0001; Table 1). Doses of the anesthetics did not differ significantly between surviving aged mice (61.4 ± 9.0 mg/kg ketamine, 6.2 ± 0.9 mg/kg xylazine) and deceased aged mice (69.3 ± 16.0 mg/kg ketamine, 6.9 ± 1.6 mg/kg xylazine; p = 0.17; Table 1).

**Discussion**

Our data clearly show that a standard dose of ketamine/xylazine with no related severe adverse effects in young mice can be detrimental in aged animals. We observed a mortality of 15% in mice with an age of approximately 2 years, although aged mice received substantially lower doses of the anesthetics per body weight. Weight and motor performance assessed before anesthesia were not able to predict outcome.

The combination of ketamine and xylazine is one of the most frequently used anesthetic regimens in rodents [11–14]. Hypotension and heart rate decrease are the major adverse reactions of ketamine/xylazine in mice, whereas respiratory functions are less affected [14]. Widely used doses of ketamine 100 mg/kg and xylazine 10 mg/kg have been shown to result in a sufficient anesthetic depth and low respiratory depression [14]. These doses have also been used for anesthesia in our mouse meningitis models since many years without causing problems concerning mortality [27, 28]. Reported doses for ketamine/xylazine in mice range from 60 to 200 mg/kg ketamine and 4–20 mg/kg xylazine [11, 12]. In the present study, we induced anesthesia by injection of 2 mg ketamine and 0.2 mg xylazine per mouse independently of the body weight. Thus, the mouse with the lowest weight (19 g) received approximately 100/10 mg/kg ketamine/xylazine; all other mice received lower doses. The mouse with the highest weight (44 g) received only 45/4.5 mg/kg ketamine/xylazine. According to their higher body weight, aged mice received significantly lower doses than young mice (only approximately 70% of the doses of young mice) which are within the lower range of reported doses or even beneath [11–14].

![Fig. 1 Mortality of young and aged mice after anesthesia with ketamine/xylazine. Kaplan-Meier curves of young mice (2 months) and aged mice (26 months) after intraperitoneal injection of 2 mg ketamine and 0.2 mg xylazine. 15.4% of the aged mice but none of the young mice died after anesthesia (log-rank test: p = 0.039).](image)

### Table 1

|                      | young (n = 26) | aged (n = 26) | p         | surviving aged (n = 22) | deceased aged (n = 4) | p       |
|----------------------|---------------|--------------|-----------|------------------------|-----------------------|---------|
| age in months (mean ± SD) | 2.14 ± 0.23   | 26.3 ± 2.15  | < 0.0001  | 26.18 ± 2.26            | 27.0 ± 1.35            | 0.49    |
| sex distribution [female: n (%)] | 17 (65%)     | 18 (69%)     | 0.77      | 15 (68%)               | 3 (75%)               | 0.79    |
| weight in g (mean ± SD)    | 23.2 ± 3.4    | 32.8 ± 5.4   | < 0.0001  | 33.3 ± 5.0              | 30.2 ± 7.5             | 0.30    |
| ketamine dose (mg/kg)      | 87.9 ± 12.0   | 62.6 ± 10.3  | < 0.0001  | 61.4 ± 9.0              | 69.3 ± 16.0            | 0.17    |
| xylazine dose (mg/kg)      | 8.8 ± 1.2     | 6.3 ± 1.0    | < 0.0001  | 6.2 ± 0.9               | 6.9 ± 1.6              | 0.17    |
Fig. 2 (See legend on next page.)
Elderly patients are undergoing surgical procedures with increasing frequency [33, 34]. They are at a relatively higher risk of perioperative mortality and morbidity compared to younger patients [4, 35]. To what extent anesthesia is causative for this, is difficult to study in humans. Guidelines recommend a 25–50% reduction of anesthetics in elderly patients [34]. However, suggested age-correction of anesthetic doses often is not sufficiently performed in clinical practice, and this failure might contribute to increased perioperative morbidity and mortality [36, 37].

**Conclusions**

The present data from our geriatric mouse model strongly corroborate the necessity of reduction of anesthetic doses and adaption of anesthetic regimens in aged individuals. Animal experiments specifically designed to compare effects of anesthetics in aged and young animals might help to identify parameters accounting for the increased susceptibility to anesthesia and to reduce the anesthesia-related perioperative mortality of aged individuals.
Abbreviations
E. coli: Escherichia coli; SD: Standard deviation

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Authors’ contributions
SS and AM performed the experiments. SS, SR, and RN analyzed and discussed the results. SS wrote the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Institute of Neuropathology, University Medical Center Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany. 2Department of Geriatrics, AEGLESION Frankfurter Diakonie Kliniken, 60431 Frankfurt am Main, Germany. 3Department of Clinical Neurophysiology, University Medical Center Göttingen, 37075 Göttingen, Germany. 4Department of Geriatrics, Evangelisches Krankenhaus Göttingen-Weende, 37075 Göttingen, Germany.

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