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Intraocular pressure in the smallest primate aging model: the gray mouse lemur

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► **To cite this version:**

Marko Dubicanac, Marine Joly, Julia Strüve, Ingo Nolte, Nadine Mestre-Francés, et al.. Intraocular pressure in the smallest primate aging model: the gray mouse lemur. *Veterinary Ophthalmology*, 2018, 21 (3), pp.319-327. 10.1111/vop.12434 . hal-01977709

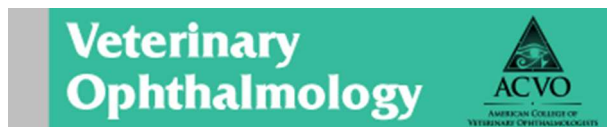
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**Intraocular pressure in the smallest primate aging model,
the gray mouse lemur**

Journal:	<i>Veterinary Ophthalmology</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Original Report
Date Submitted by the Author:	n/a
Complete List of Authors:	Dubicanac, Marko; Tierarztliche Hochschule Hannover, Institute of Zoology Joly, Marine; Centre for Comparative and Evolutionary Psychology, King Henry Building Strueve, Julia; Tierarztliche Hochschule Hannover, Small Animal Clinic of the University of Hanover Nolte, Ingo; Tierarztliche Hochschule Hannover, Small Animal Clinic of the University of Hanover Verdier, Jean-Michel; Université Montpellier 2, Inserm U1198 Frances-Mestre, Nadine; Université Montpellier 2, Inserm U1198 Zimmermann, Elke; Tierarztliche Hochschule Hannover, Institute of Zoology
Keywords:	intraocular pressure, tonometer, reference value, mouse lemur, primate, aging

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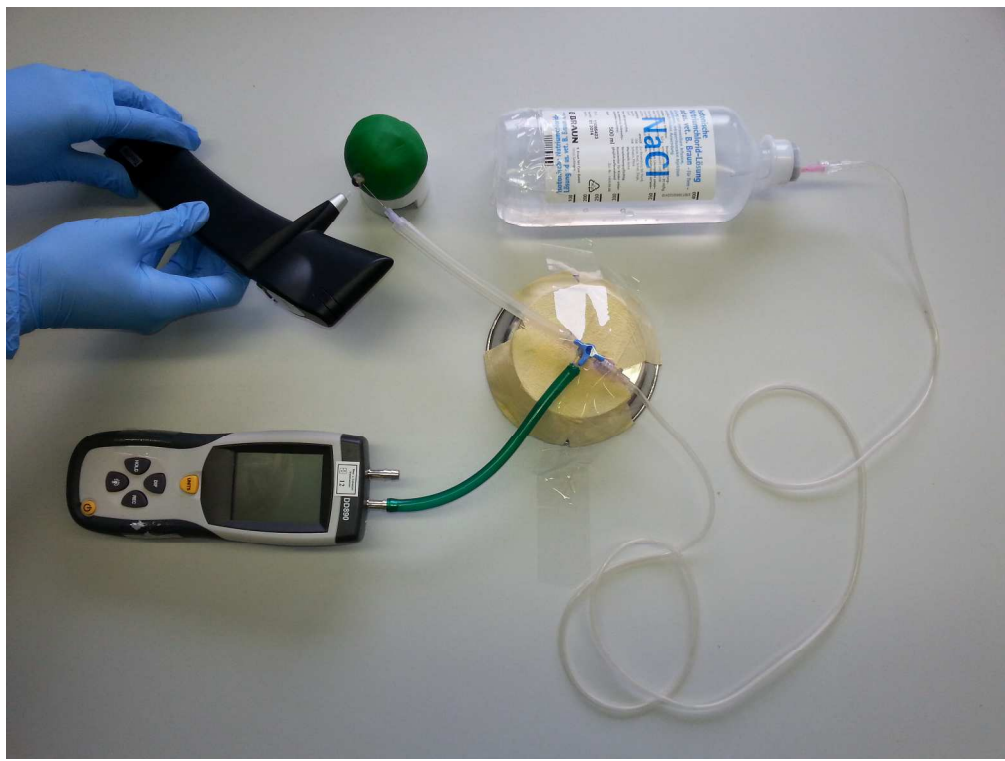


Figure 1. Setup of the manometrical investigation.
1151x863mm (72 x 72 DPI)

Review

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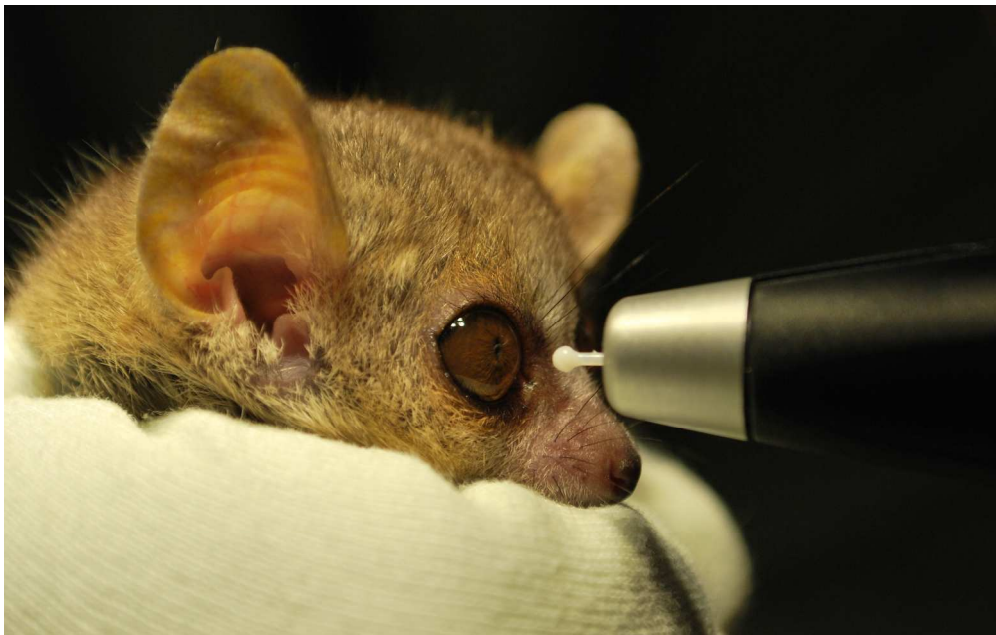


Figure 2. Investigation of a 2 year old mouse lemur with the TonoVet®.
283x179mm (300 x 300 DPI)

Review

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Figure 3. Investigation of a 2 year old mouse lemur with the TonoPen™
1024x685mm (96 x 96 DPI)

Review

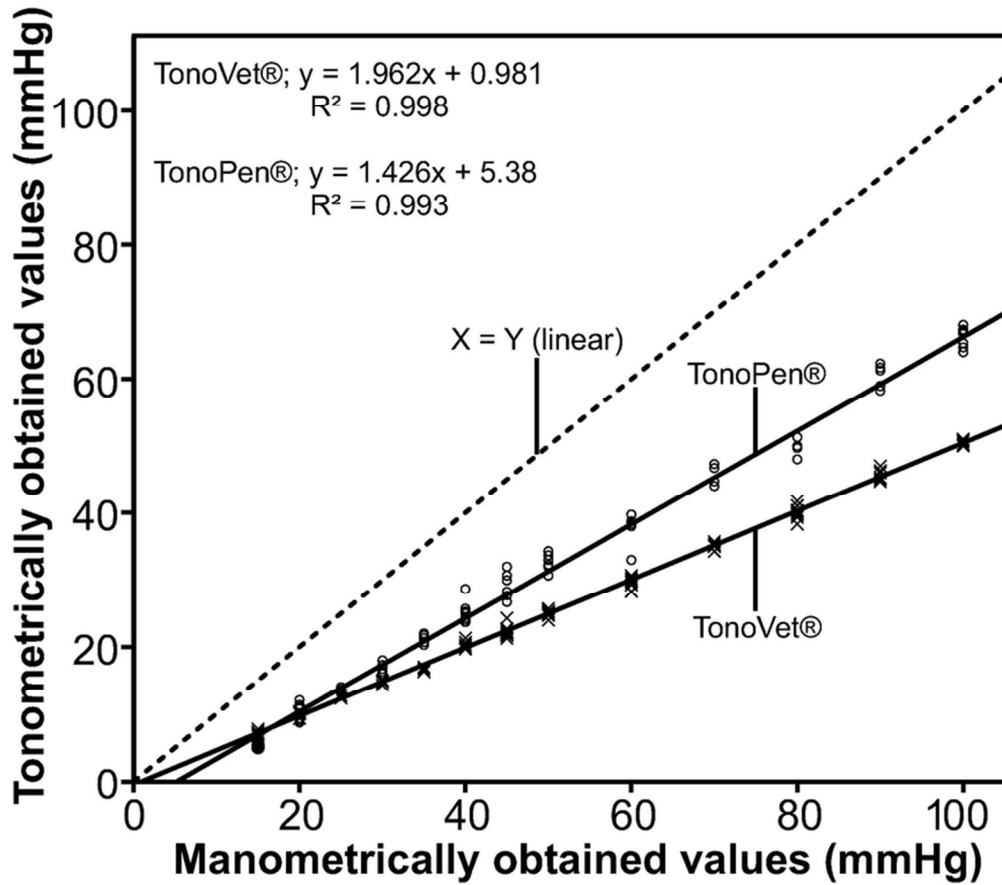


Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical control. Manometrically determined IOP for the eight eyes of four animals are represented on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot represents the mean from three measurements per step/per eye. The dashed line represents the regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.

73x64mm (300 x 300 DPI)

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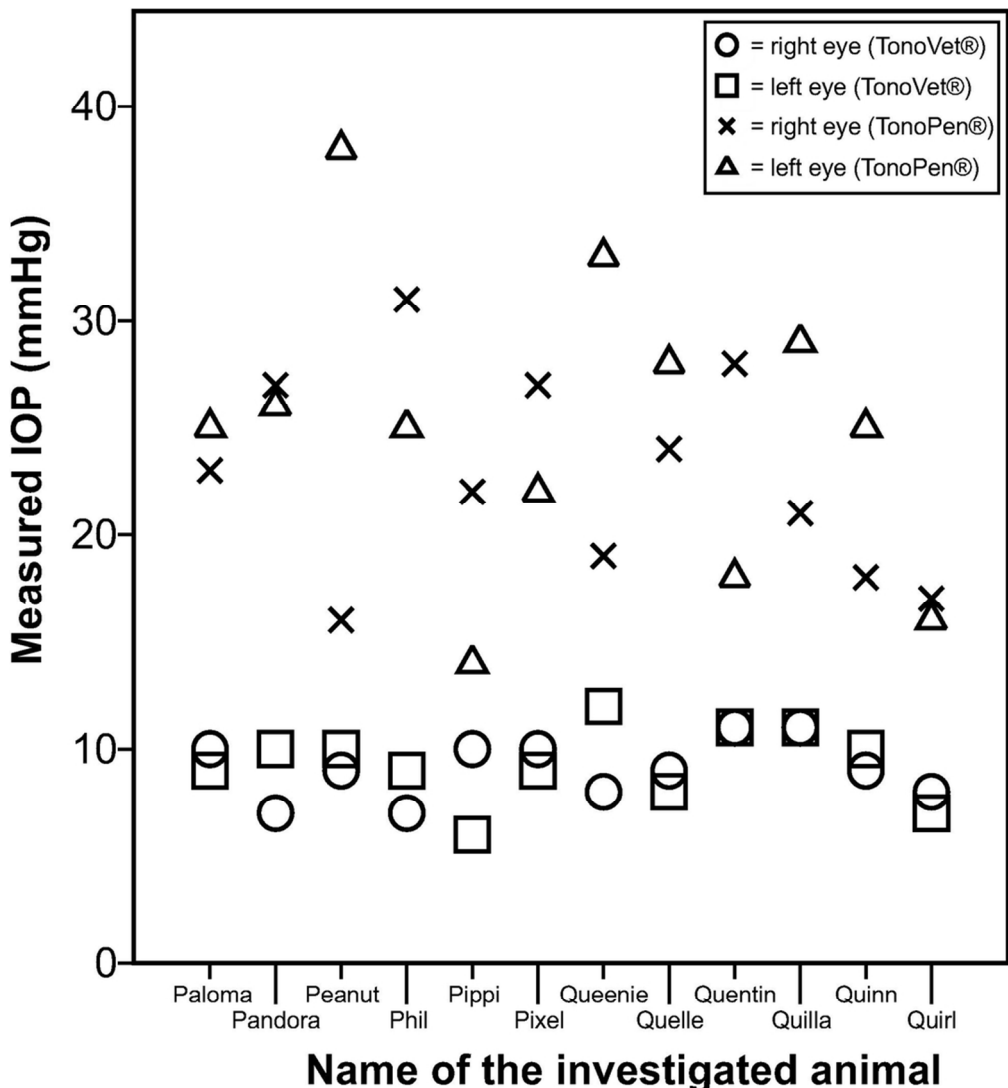


Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in 12 animals. The results show high variation in measurements for the TonoPen™ and much more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Tab. 1).
91x99mm (300 x 300 DPI)

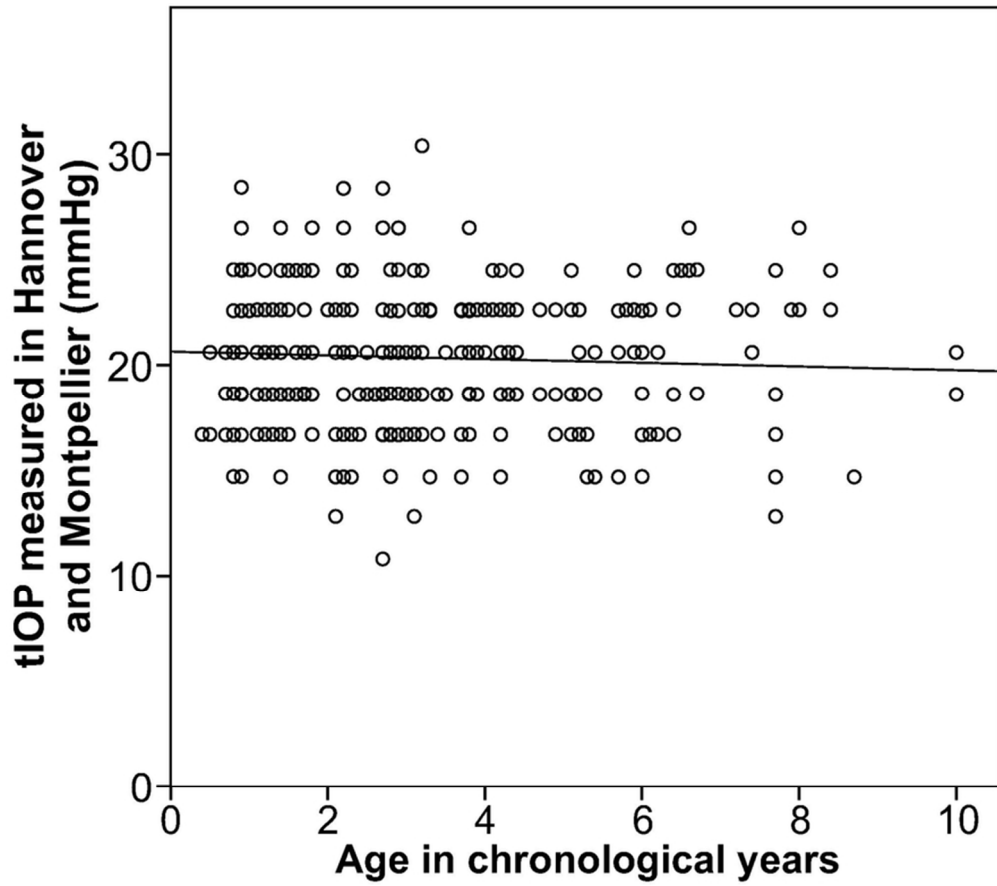


Figure 6. Relation between tIOP values and age in healthy mouse lemur eyes. This scatterplot shows the tIOP values for all measured healthy animals from Hannover and Montpellier (N = 258) on the y-axis in relation to age on the x-axis. The decrease in IOP is statistically not significant ($p = 0.077$, $r_s = -0.110$).
74x65mm (300 x 300 DPI)



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3 **1 Intraocular pressure in the smallest primate aging model, the gray mouse lemur**
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3 Suggested running title: Intraocular pressure in aging mouse lemurs

4
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3 26 **Abstract**
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7 28 **Objective:** The aim of this study was to assess the practicability of common tonometers used
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10 29 in veterinary medicine for rapid IOP screening, to calibrate IOP values gained by the
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12 30 tonometers and to define a reference IOP value for the healthy eye in a new primate model
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14 31 for aging research, the gray mouse lemur.
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16 32 **Studied animals & Procedures:** TonoVet® and the TonoPen™ measurements were
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18 33 calibrated manometrically in healthy enucleated eyes of mouse lemurs euthanized for
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20 34 veterinary reasons. For comparison of the practicability of both tonometers as a rapid IOP
21
22 35 assessment tool for living mouse lemurs, the IOP of 24 eyes of 12 hand-fixed animals (six
23
24 36 males and six females) was measured. To define a standard reference value for IOP in 258
25
26 37 healthy mouse lemurs, two of the largest colonies in the world were examined using the most
27
28 38 practicable tonometer.
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31 39 **Results:** IOP measurements for the TonoVet® can be corrected by the formula: $y = 0.981 +$
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33 40 $(1.962 * \text{TonoVet}^{\circledR} \text{ value})$, for the TonoPen™ by $y = 5.38 + (1.426 * \text{TonoPen}^{\text{TM}} \text{ value})$. The
34
35 41 calibrated IOP for a healthy mouse lemur eye is 20.3 ± 2.85 mmHg. The TonoVet® showed
36
37 42 advantages in practicability, e.g. small corneal contact area, short and painless corneal contact
38
39 43 and time. IOP measurements of healthy mouse lemur eyes were not affected by age, sex, eye
40
41 44 side or colony.
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44 45 **Conclusion:** Tonometry using TonoVet® is the more practicable assessment tool for IOP
45
46 46 measurement of the tiny eyes of living mouse lemurs. Pathological deviations can be
47
48 47 identified based on the described reference value.
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52 49 **Key words:** intraocular pressure, tonometer, reference value, mouse lemur, primate, aging
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3 51 **Introduction**
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5 52 Mouse lemurs belong to the smallest living primates worldwide. (1) Due to this fact, their
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7 53 maintenance and breeding is more cost-efficient than in larger primate species and they are
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9 54 also not known for spreading zoonotic diseases which makes them an extraordinary primate
10
11 55 model for research. Additionally mouse lemurs have a life expectancy which is much shorter
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13 56 than in other non-human primate aging models with about 8 years in the wild and up to 18.5
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15 57 years in captivity. (2, 3) The genome of mouse lemurs has recently also been sequenced by
16
17 58 the Broad Institute (GenBank accession number ABDC00000000). Besides of the importance
18
19 59 of mouse lemurs for biomedical as well as aging research, (4-9) they are also important for
20
21 60 evolutionary research, based on their high and cryptic species diversity, their uneven
22
23 61 distribution and their flexible adaptations to their natural habitats. (10)
24
25 62 Mouse lemurs are nocturnal and have relative small absolute eye sizes with 9.4 mm in
26
27 63 diameter. (11, 12) Aged gray mouse lemurs were reported to suffer from different eye
28
29 64 diseases such as cataract, retinal atrophy and buphthalmia, an abnormal enlargement of the
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31 65 eyeball. (13) Whether this malformation was due to glaucoma, which causes ocular
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33 66 hypertension, still need to be clarified though. Because of the difficulty in handling non-
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35 67 anesthetized animals, however, IOP was never studied in mouse lemurs before. Applanation
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37 68 and rebound tonometry are commonly used in veterinary medicine to determine IOP in
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39 69 domestic animals such as dogs, (14, 15) cats, (16-18) and birds (19, 20) as well as in
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41 70 laboratory animals such as rats, (21, 22) rabbits (23) and macaques. (24, 25) Since factory
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43 71 settings for TonoPen™ and TonoVet® are only available for common species in the
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45 72 veterinary clinics such as dogs, cats and horses, for uncommon species it is necessary to
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47 73 calibrate measurements by manometry to get a true IOP (tIOP) before defining standard
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49 74 reference IOP values for a given species. (see e.g. rabbits, (26) birds (20) and macaques. (25))
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3 75 The TonoPen™ is an applanation tonometer often used for intraocular measurements in
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5 76 veterinary medicine. (14, 16, 21) IOP measurement gives an indirect assessment of the IOP
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7 77 by using the Imbert-Fick law. (27) It measures the counter pressure that is necessary to flatten
8
9 78 a thin membrane surrounding a sphere filled with liquid. Its use in dogs and cats is easy and
10
11 79 fast but requires a local anaesthesia of the cornea.

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14 80 The TonoVet® is a rebound tonometer based on a patented measurement system which uses
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16 81 a small, disposable probe which is brought into contact with the cornea. (17, 18, 20, 24, 28)

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18 82 The probe is rebounding with a determined speed, correlating to the IOP. The higher the IOP
19
20 83 is, the higher the speed of the return-bounce. Its use is easy and fast and requires no local
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22 84 anaesthesia.

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25 85 In this study we applied TonoPen™ and TonoVet® as rapid IOP assessment tools to the gray
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27 86 mouse lemur, to

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30 87 1. calibrate IOP measurements of the tonometers by manometry,
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32 88 2. assess the practicability of the tonometers to measure the IOP of mouse lemurs' eyes
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34 89 in-vivo to screen colonies,
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36 90 3. apply the most practicable technique for screening IOP in two of the world's largest
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38 91 colonies, to investigate the effect of eye position, sex, colony and age on IOP and
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40 92 establish a reference value for IOP.

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3 100 **Methods**

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7 102 **Animals and maintenance**

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9 103 Mouse lemurs (*Microcebus murinus*) tested in this study belonged to two licensed breeding
10 104 colonies housed at the Institute of Zoology at the University of Veterinary Medicine
11 105 Hannover (for details in housing conditions see; (29) Hannover breeding licence number
12 106 42500/1H) and the University of Montpellier 2 (Agreement N^{br} ≠ C-34-172-23). Out of 349
13 107 investigated animals 258 animals, which showed no ocular pathologies, have been used for
14 108 analysis, 75 (38 females; 37 males) from Hannover and 183 (101 females; 82 males) from
15 109 Montpellier, ranging from 0.5 to 10 years. All animals were born in captivity. Since mouse
16 110 lemurs are nocturnal, the captive animals were maintained under artificial light conditions
17 111 with a reversed light cycle. Additionally, animals in Montpellier are maintained under an
18 112 accelerated photoperiodic regime. This means that the photoperiodically triggered
19 113 reproductive “year” lasted 8 instead of 12 months. It has been shown that these conditions
20 114 accelerate aging processes in gray mouse lemurs by the factor 1.5. (30-32)

21 115

22 116 **Ophthalmologic investigation**

23 117 Handling for ophthalmological examinations was similar to the weekly caretaker handling of
24 118 the animals resulting in reduced stress for the lemurs. All examinations were conducted at the
25 119 end of the sleeping period/beginning of the activity period to minimize disturbances of the
26 120 animal’s activity. All procedures applied in this study were licenced by the respective
27 121 authorities (Hannover licence number, 33.9-42502-05-11A200, LAVES to Elke
28 122 Zimmermann; Montpellier licence number, 34-124 to Jean-Michel Verdier).

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3 124 Both eyes of a lemur were investigated with a slit-lamp bio-microscope (SL-14; Kowa,
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5 125 Eickemeyer, Germany) and indirect ophthalmoscope (Omega 100; Heine, Ettenheim,
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7 126 Germany) to determine potential eye pathologies with a possible effect on IOP or corneal
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9
10 127 consistence. To get a view on the lens and retina, mydriatic eye-drops (Mydrum®, Chauvin
11
12 128 ankerpharm GmbH, Berlin, Germany) were used to widen the pupil.

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15 16 130 **1. Manometry**

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18 131 We determined the IOP value of an enucleated eye with the TonoVet® and TonoPen®,
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20 132 respectively, at manometrically defined IOP pressure steps (DD-890, ATP Messtechnik
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22 133 GmbH, Ettenheim, Germany). The used manometer was calibrated by the bureau of standards
23
24 134 in Hannover (Mess- und Eichwesen Niedersachsen Betriebsstelle Eichamt Hannover,
25
26 135 Goethestraße 44, 30169 Hannover). The pressure measured by the manometer in this setup
27
28 136 (including the pressure in the examined eye) will be labelled as the true IOP (tIOP). The
29
30 137 values in the more relevant sector for clinical use between 5 mmHg and 50 mmHg have been
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32 138 taken in steps of 5 mmHg ± 0.1 mmHg. Between 50 mmHg and 100 mmHg measurements
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34 139 were taken in steps of 10 mmHg ± 0.1 mmHg.

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36 140 Eight healthy eyes of four animals euthanized for veterinary reasons (incurable pathologies)
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38 141 were enucleated transconjunctivally immediately after euthanasia. These eyes were called
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40 142 healthy since they were found inconspicuous and showed no signs of pathological disease
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42 143 according to an ophthalmological investigation performed not more than 6 months before.

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44 144 After enucleation, the eyes were stored in 0.9% NaCl solution at 6°C for up to a maximum of
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46 145 4 hours before measurement. A small bowl of dough was adjusted to ensure the fixation of
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48 146 the enucleated eye. The cannula (24 G, length 25 mm, B. Braun Melsungen AG, D-34209)
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50 147 was inserted transsclerally into the vitreous and was not moved or reinserted while taking the
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52 148 measurement. The pressure was constant and measured values showed no fluctuation.

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3 149 Minimal leakages were observed sporadically, but sealed by themselves with higher pressure.
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5 150 A three-way stopcock was connected with the cannula, the manometer and a NaCL solution
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7 151 reservoir via three silicon tubes. Pressure was adjusted by changing the height of the NaCl
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9 152 solution reservoir. The whole system was opened all the time to avoid fluctuations. (see Fig.
10
11 153 1) Once the manometer displayed a constant pressure, the pressure was measured using the
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13 154 TonoVet® and TonoPen™. Each complete measurement by the TonoVet® represented the
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15 155 mean of four single values, for the TonoPen™ the mean consists out of four to five single
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17 156 values. The measurement was carried out until three complete measurements per eye were
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19 157 successfully obtained.
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27 160 **2. In-vivo application of TonoPen™ and TonoVet®**

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29 161 The tonometers TonoPen™ and TonoVet® were used to measure IOP in the eyes of 12
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31 162 young mouse lemurs (6 males, 6 females; aged between 2 and 3 years; Colony Hannover).
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33 163 Both eyes of these mouse lemurs were inconspicuous and showed no signs of pathological
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35 164 diseases according to an ophthalmological investigation performed one day before.
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37 165 Measurements of the TonoVet® and TonoPen™ have been taken 24 hours after the
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39 166 ophthalmological investigation to minimize influences of the mydriatic eye-drops on the IOP.
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41 167 Each animal was investigated on both eyes until a successful measurement with the
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43 168 respective tonometer has been achieved. The successful measurement is indicated by an
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45 169 acoustical signal.
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3 174 **2.a Rebound tonometry (TonoVet®)**
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5 175 Six single values of the IOP were taken per eye by the TonoVet® tonometer (TonoVet®;
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7 176 ICare, Finland Oy). The TonoVet® then automatically deleted the lowest and highest value
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9 177 and calculated the mean out of the remaining four values. Only successfully completed
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11 178 measurements displaying a mean on the TonoVet® were recorded. One successfully
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13 179 completed measurement per animal was taken. For all 12 animals no anaesthesia and no
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15 180 forced fixation of the eyelids were necessary (see Fig. 2).
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21 182 **2.b Applanation tonometry (TonoPen™)**
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23 183 The TonoPen™ tonometer (TonoPen™; Reichert® Technologies, Eickemeyer, Germany)
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25 184 was used to measure IOP of the same 12 animals as for the TonoVet®. Before measurements
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27 185 were taken, the eyes were locally anaesthetized with eye-drops (Proparacain-POS® 0.5%).
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29 186 To prevent a reflective wink of the eyelids of an animal, the examiner had to fix the eyelids
30
31 187 with his fingers in open position (see Fig. 3). Between four and five single values per eye
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33 188 were necessary for the TonoPen™ to calculate a mean. Only successfully completed
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35 189 measurements displaying a mean on the TonoPen™ were recorded. One successfully
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37 190 completed measurement per animal was taken.
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43 192 **3. Determination of IOP in two colonies**
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45 193 To determine a reference value for the healthy mouse lemur eye, a large sample size is
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47 194 required.
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49 195 241 animals in Montpellier and 108 animals in Hannover have been investigated. For the
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51 196 determination of the IOP the TonoVet® was used. The animals were investigated at the end
52
53 197 of the sleeping/beginning of the activity period. For Montpellier the time for investigation
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55 198 ranged from 09:00 a.m. – 03:00 p.m. (beginning of activity period at 12:00 a.m. for all
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3 199 animals), for Hannover from 09:00 a.m. – 05:00 p.m. (beginning of activity period at 10:00
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5 200 a.m., 12:00 a.m. or 02:00 p.m. respectively, according to the room). After IOP measurement,
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7 201 an ophthalmological investigation was performed for each animal as described in the
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10 202 paragraph ophthalmologic investigation to select animals with healthy eyes for this study. 58
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12 203 animals for Montpellier and 33 animals for Hannover showing eye malformations were
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14 204 thereby excluded from further analysis.
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18 207 **Data analysis**

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25 209 1. Manometric calibration of the IOP measured by TonoVet® and TonoPen™ in
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27 210 enucleated eyes

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30 211 For the manometric calibration of the TonoVet® and TonoPen™ IOP measurements, we
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32 212 performed a regression analysis out of the eight means (= eight eyes) per step mmHg per
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34 213 instrument.
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38 215 2. Comparison of IOP between TonoPen™ and TonoVet®

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40 216 For the in-vivo comparison of applanation (TonoPen™) and rebound (TonoVet®) tonometry,
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42 217 we calculated the mean, range, standard deviation and median IOP for each instrument out of
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44 218 the measurements of the 24 eyes of the 12 animals. For the TonoVet® tonometer and the
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46 219 TonoPen™ tonometer, we compared measured IOP values between left and right eyes using
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48 220 the paired t-test and measured IOP values between sexes using the unpaired t-test since the
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50 221 values followed a normal distribution. The IOP values obtained by the TonoPen™ and
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52 222 TonoVet® were compared using the Wilcoxon-signed-rank test.
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3 224 3. Comparison of IOP across colonies
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5 225 To assess the effect of sex and eye position, IOP values (using TonoVet®; $N_{\text{totalanimals}} = 258$,
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7 226 $n_{\text{totaleyeyes}} = 516$; Hannover, $N = 75$; Montpellier, $N = 183$) obtained for each eye and animal per
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9
10 227 colony were compared between the left and right eye and between sexes using the Wilcoxon-
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12 228 signed-rank test and Mann-Whitney-U test, respectively.
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14 229 If findings within colonies did not reveal a significant effect of eye position or sex, we used
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16 230 the median value of an animal per colony for further statistical analysis. To explore the effect
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18 231 of colony, we compared IOP values between colonies using the Mann-Whitney-U test.
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20 232 The effect of age on IOP was analysed using a Spearman-Rank correlation. For the colony of
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22 233 Montpellier, the cycle age was multiplied by the factor 1.5 to calculate the chronological age
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24 234 in years. To define the reference IOP value for a healthy mouse lemur eye, we calculated the
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27 235 mean, range, standard deviation and median of the IOP of the healthy eye of both colonies.
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1
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3 249 **Results**

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7 251 1. *In vitro calibration of the rebound (TonoVet®) and applanation (TonoPen™)*

8
9 252 *tonometry*

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11 253 We found a linear correlation between either the rebound tonometry and direct manometry or
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13 254 applanation tonometry and direct manometry (see Fig. 4; n = 8 eyes; N = 4 mouse lemurs).

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15 255 The regression analysis showed consistent linear underestimation of IOP by the TonoVet®
16
17 256 and TonoPen™. From 15 mmHg up to 100 mmHg, the regression analysis showed that the
18
19 257 measured IOP (mIOP) for TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$)
20
21 258 can be corrected by using the function $tIOP = 0.981 + (1.962 * mIOP)$ and for TonoPen™ (F
22
23 259 $= 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) by using the function $tIOP = 5.38 +$
24
25 260 $(1.426 * mIOP)$. In both tonometers it was not possible to obtain values below 15 mmHg.

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27 261 From 15 mmHg up to 100 mmHg the TonoVet® constantly underestimated the tIOP by half.
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29 262 While the TonoPen™ almost measured the same values at 20 mmHg as the TonoVet®, the
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31 263 measured values start slightly to increase compared to the TonoVet® reaching around 66%
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33 264 of the tIOP at 100 mmHg.

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41 268 2. *In-vivo IOP measurements with TonoPen™ and TonoVet®*

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45 270 A comparison of IOP values between the left and right eye for each instrument showed no
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47 271 significant difference between eye side (TonoVet®, paired t-test, $N = 12$, $t = -0.538$, $p =$
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49 272 0.601 ; TonoPen™, paired t-test, $N = 12$, $t = -0.794$, $p = 0.444$).

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3 273 No difference between sexes was found for IOP neither for the TonoVet® (unpaired t-test,
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5 274 $N_{\text{total}} = 24$, $N_{\text{males}} = N_{\text{females}} = 6$, $t = -0.130$, $p = 0.292$) nor for the TonoPen™ (unpaired t-test,
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7 275 $N_{\text{total}} = 24$, $N_{\text{males}} = N_{\text{females}} = 6$, $t = -0.340$, $p = 0.198$). Thus, we have not differentiated between
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9
10 276 sexes in all further analyses.

11 277 The mean IOP for the TonoVet® tonometer was 9.21 ± 1.53 mmHg, the median was 9.0
12
13 278 mmHg and the range 6-12 mmHg, the tIOP was 19.03 ± 2.24 mmHg.

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16 279 For the TonoPen™ tonometer the mean was 23.83 ± 5.89 mmHg, the median 24.5 mmHg
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18 280 and the range 14-38 mmHg with a tIOP of 39.36 ± 5.1 mmHg. (see Tab. 1)

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21 281 IOP values measured by the TonoVet® and the TonoPen™ differed significantly (Wilcoxon-
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23 282 test, $N = 24$, $T = 0.00$, $n = 24$, $p = < 0.001$). The comparison of measured values between
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25 283 instruments showed a higher estimation of the average IOP for the TonoPen™. The average
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27 284 value for the TonoPen™ is more than twice as high as the average value estimated by the
28
29 285 TonoVet®. Measurements with the TonoPen™ in the same animal for the left and right eye
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31 286 show high variability, while the values obtained with the TonoVet® are much more
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33 287 consistent e.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left
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35 288 eye 10 mmHg, right eye 9 mmHg). (see Fig. 5)

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40 290 Several problems occurred in the application of the TonoPen™ in the examiner-animal
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42 291 context. The tip of the TonoPen™ was large in comparison to the small eyes of the mouse
43
44 292 lemurs causing reflective winks of the animal because of contact to the animals' eyelashes. It
45
46 293 was not possible to fixate the animals' eye-lids without causing indirect pressure on the eye-
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48 294 bulb. Besides, the examiner had to protect himself against bites since the hand which was
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50 295 fixating the animals' eye-lids was hazardously close to the animals' mouth and sharp teeth.
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52 296 The time that was necessary to complete one successful measurement per animal varied
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54 297 between 5 and 10 minutes (without waiting time for the applied anaesthetizing eye-drops).
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5 299 When using the TonoVet® for IOP measurement, the animals showed no visible reaction
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7 300 when the probe touched the animals' cornea, furthermore almost no reflective wink was
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9 301 visible. Since no forced fixation of the animals' eye-lids was necessary, bites never occurred.
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11 302 The investigation lasted on average only 30 seconds.
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18 305 *3. Comparison of IOP between two mouse lemur colonies and the definition of a*
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21 306 *reference IOP value for the gray mouse lemur*
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25 308 Both colonies have been investigated using the TonoVet®. A comparison between measured
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27 309 IOP values in the left and right eye for each colony showed no significant differences
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29 310 (Hannover, Wilcoxon-test, $N = 75$, $T = 22.05$, $n = 47$, $p = 0.361$; Montpellier, Wilcoxon-test,
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31 311 $N = 183$, $T = 55.27$, $n = 113$, $p = 0.336$).

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34 312 No difference between sexes was found for IOP (Hannover, Mann-Whitney-test, $N_{\text{total}} = 150$,
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36 313 $N_{\text{males}} = 37$ eye-pairs, $N_{\text{females}} = 38$ eye-pairs, $U = -1.314$, $p = 0.189$; Montpellier, Mann-
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38 314 Whitney-test, $N_{\text{total}} = 366$, $N_{\text{males}} = 82$ eye-pairs, $N_{\text{females}} = 101$ eye-pairs, $U = -1.552$, $p =$
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40 315 0.121). Thus, sexes were not further differentiated in further analyses.
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42
43 316 The comparison of IOP values between both colonies showed no significant difference
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45 317 (Mann-Whitney-test, $N_{\text{total}} = 516$, $N_{\text{Hannover}} = 75$ eye-pairs, $N_{\text{Montpellier}} = 183$ eye-pairs, $U = -$
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47 318 0.230 , $p = 0.818$). Based on that, we did not further differentiate between colonies for
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49 319 subsequent analysis.

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51 320 The effect of age on IOP was assessed by a Spearman-Rank correlation. No significant
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53 321 correlation between chronological age and IOP was revealed ($p = 0.418$, $r_s = -0.036$, $N = 516$,
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55 322 see Fig. 6). Thus, age has not affected IOP in the healthy eye for the investigated age-span.
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3 323 Based on that a reference value for IOP measured with the TonoVet® was calculated with a
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5 324 mean of 9.87 ± 1.56 mmHg, a median of 10 mmHg and a range of 5-15 mmHg. Using the
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7 325 regression function the calculated mean for healthy mouse lemur eyes is $tIOP = 20.3 \pm 2.85$
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9 326 mmHg.
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3 348 **Discussion**

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7 350 This is the first study measuring the IOP of mouse lemurs. Our findings suggest that the
8
9 351 TonoVet® is the most suitable tool (compared to the TonoPen™) for rapid IOP screening of
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11 352 the tiny eyes of this smallest bodied primate. Results showed that IOP in the clinically
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13 353 healthy eye is not affected by age, sex, eye side (left or right). Thus, a reference value for IOP
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15 354 could be defined based on a large sample-size of more than 250 individuals for this novel
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17 355 primate model for aging research.
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23 357 **Calibration of IOP using manometry**

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25 358 Every tonometer has to be calibrated for each species specifically due to the fact of different
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27 359 corneal attributes, especially corneal thickness, to get the tIOP values. (33, 34) IOP
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29 360 measurements using eyes persisting in the eye socket in ex-vivo as well as enucleated eyes
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31 361 revealed no significant differences between these procedures. (35) Manometric in-vivo
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33 362 measurement of IOP is, however, known to potentially cause damage at intraocular structures
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35 363 and pain, thus for ethical reasons we decided to base our manometric investigation on
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37 364 enucleated eyes of animals which died for natural reasons or were euthanized due to
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39 365 incurable diseases which had no impact on IOP.
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43 366 Our manometric calibration for the TonoVet® and TonoPen™ showed a consequent linear
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45 367 underestimation of the tIOP for both instruments which can be corrected by the established
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47 368 regression functions. For a fast clinical interpretation of the measurements displayed on the
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49 369 TonoVet® the values can be multiplied by 2. For the TonoPen™ a similar but not as easy to
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51 370 use formula is: $tIOP = (1.5 * \text{measured value}) + 5$.

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53 371 A comparable underestimation of the IOP measured by the TonoVet® and TonoPen™ was
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55 372 reported for dog eyes. (15) This underestimation was explained by corneal specification
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3 373 especially corneal thickness, calibration-standards and use by different examiners. For our
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5 374 study, the same examiner performed the measurements so that the effect of the examiner on
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7 375 the measured IOP values was minimized whereas both corneal specification and different
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9 376 calibration standards between TonoVet® and TonoPen™ are likely to explain the
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11 377 underestimation of IOP values. In humans a significant effect on IOP measurements caused
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13 378 by different thickness in different corneal areas using the rebound tonometry has already been
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15 379 shown: higher values were determined when corneal thickness was higher. (33, 34) Our
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17 380 measurements were taken at the center of the cornea to minimize this effect. We expect that
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19 381 central corneal thickness in mouse lemurs is relatively thin, which would explain the linear
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21 382 underestimation. Further investigations on corneal thickness of mouse lemurs e.g. with an
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23 383 ultrasound pachymeter are necessary to investigate this in more detail.
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385 **Practicability of TonoPen™ or TonoVet® to screen the IOP of mouse lemur eyes**

386 The practical value of a tonometer is as important as the calibration. Thus, which of the
387 commonly used tonometers in veterinary science, TonoPen™ or TonoVet®, is the most
388 suitable tool to screen tiny eyes of a large number of mouse lemurs in colonies on a regular
389 basis? The eyes of the gray mouse lemur have only a diameter of 9.4 mm. (11) Thus, a
390 previous ophthalmological study in the gray mouse lemur had difficulties in using
391 applanation tonometry in non-anesthetized animals. (13) We applied both the TonoPen™ and
392 the TonoVet® for a subgroup of non-anesthetized animals to assess the practicability of these
393 instruments. We showed that both tonometers can be applied, but that there are huge
394 differences in practicability and ethical justifiability. The investigation with the TonoPen™
395 required a much longer time (up to 10 minutes) to assess the IOP of an animal compared to
396 TonoVet® (up to 30 seconds). The most time consuming issue emerged by the high number
397 of failed IOP measurements (indicated by an alarm-signal of the TonoPen™). The failure in

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3 398 measurement was due to the fact that the veterinarian had to pay attention to exerted pressure,
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5 399 contact-area and animal position while measuring the IOP. In contrast, unsuccessful
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7 400 measurements (indicated by an alarm-signal) were quite rare for the TonoVet®. Furthermore,
8
9 401 the small eye-bulbs of the mouse lemurs make it necessary to fixate the eye-lids manually
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11 402 and eventually causing pressure on the eye-bulb whereas no fixations of the animal's eye-lids
12
13 403 were necessary for the TonoVet®. The animal itself must be fixated much stronger when
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15 404 using the TonoPen™ causing systemical hypertension and consecutively higher IOP.
16
17 405 Consequently, our experience using the TonoPen™ supports Beltran et al. (13) that the use of
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19 406 this instrument to measure IOP in the tiny eyes of mouse lemurs is problematic. The
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21 407 extensive manipulation which is necessary prevents the determination of physiological
22
23 408 expected IOP values. Usually physiological IOP ranges from 15-23 mmHg e.g. in humans,
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25 409 (36) dogs, (14) cats, (18) horses, (28) rabbits, (23) rats (21) and macaques. (37) (see Tab. 2)
26
27 410 Therefore non-physiologically high IOP as measured with the TonoPen™ in mouse lemurs
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29 411 ($tIOP = 39.36 \pm 5.1$) may be the result of stress, high systemic blood pressure and unintended
30
31 412 pressure on the bulbus questioning the ethical justifiability of this method.
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33 413 Other positive effects of the TonoVet® were that IOP of non-anesthetized animals can be
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35 414 measured rapidly and without any visible harm for the measured animal. Furthermore a
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37 415 veterinarian can standardize the measurement quickly and get fast routinisation. The
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39 416 TonoVet® showed satisfying results concerning reproducibility with a relatively small
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41 417 variation comparable to those of other studies using larger mammals such as rhesus macaques
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43 418 (25) or rabbits. (26) All in all, based on these findings we recommend the TonoVet® as a
44
45 419 suitable IOP assessment tool for rapid screening of the eyes in non-anaesthetized mouse
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47 420 lemurs.
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3 423 **Definition of a reference IOP for the healthy gray mouse lemur eye**
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5 424 A reliable mean for IOP in mouse lemurs requires a large sample-size. To enlarge our
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7 425 sample-size we have analyzed if eye side (left or right), sex or age had any influence on IOP
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9 426 in mouse lemurs. Our study showed no significant differences between eye side and sex and
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11 427 no statistically significant correlation between age and IOP. The slight decrease of IOP
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13 428 between the age of 0.5 and 10 years was even smaller than the value of the determined
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15 429 standard deviation. Animals of ten years and older were excluded due to diagnosed
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17 430 pathologies.
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20 431 Our screening analysis of the two colonies, Hannover and Montpellier, included 258 animals
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22 432 with 516 healthy eyes in total and showed a tIOP of 20.3 ± 2.85 mmHg. This value matches
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24 433 quite well to the IOP range of 15-23 mmHg reported from humans (36) and mammals of
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26 434 veterinary and biomedical importance (see Tab. 2) such as dogs, (14) cats, (18) horses, (28)
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28 435 rabbits, (23) rats (21) and macaques. (37) Since these mammals differ largely in size, activity
29
30 436 and phylogeny, IOP seems to be independent from these factors.
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34 437 Circadian rhythm is also described to affect IOP, e.g. in cats, rabbits and Tibetan monkey.
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36 438 (23),(38),(39) To minimize this effect, we always determined IOP at the beginning of the
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38 439 animal's activity period.
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40 440 Our study showed no correlation between age and IOP in the healthy mouse lemur eye
41
42 441 comparable to e.g. Tibetan monkeys. (39) Age-effects on IOP in animals and humans are
43
44 442 ambiguous. Whereas investigations performed in rhesus monkeys and dogs showed a
45
46 443 decrease in IOP with age (37),(14) studies in humans revealed both an increase and a
47
48 444 decrease depending on the tested populations. (40),(36),(41) High blood pressure, obesity and
49
50 445 other vascular deficiencies were discussed as explanations. (42-44) Consequently we
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52 446 postulate that physiological status and vascular conditions were healthy in our studied mouse
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54 447 lemur population.
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3 448 It has to be taken into account that for our study we considered only ophthalmological
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5 449 inconspicuous animals. Pathologies influencing the state of health of the eye and leading to
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7 450 glaucoma or higher intraocular pressure have to be considered in follow-up studies.
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12 13 14 452 **Conclusion**

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16 453 To conclude, we demonstrated the practicability, usefulness and reliability of the TonoVet®
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18 454 as a powerful tool for screening IOP in mouse lemurs, a novel primate model for human
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20 455 aging research. Average IOP of healthy mouse lemur eyes is not affected by eye side, sex and
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22 456 colony and does not correlate with age. Furthermore, the value of IOP of mouse lemurs
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24 457 coincides with those of other mammals. For future studies using this smallest-bodied primate
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26 458 aging model, our findings are an important foundation to disentangle peripheral from central
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28 459 pathologies.
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34 463 **Software for statistical analysis**

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36 464 The statistical analysis was performed using SPSS 22.0 for Windows. Significance level was
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38 465 set at $P = 0.05$.
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43 468 **List of Abbreviations**

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45 469 IOP: Intraocular pressure; tIOP: true intraocular pressure; mIOP: measured intraocular
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3 472 **Competing interests**
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5 473 The authors declare that they have no competing interests.
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10 475 **Authors' contributions**
11

12 476 MD, MJ, EZ, IN, JS have conceived, coordinated and designed the study. Manometrical data
13
14 477 was acquired by MD. Data from the screening of both colonies was acquired by MD and MJ.
15
16 478 Statistical analysis was conducted by MD. All authors contributed in drafting, reading and
17
18 479 approving the final manuscript.
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20 480

21
22 481 **Acknowledgements**
23

24 482 We are grateful to all caretakers and assistants who helped collecting data: Lisabelle Früh,
25
26 483 Iris Grages, Johanna Samtlebe, Sönke von den Berg, Elisabeth Engelke, Jennifer Brunke,
27
28 484 Kathrin Röper, Annette Klaus, Eva Schuster, May Hokan, Sabrina Linn, Sylvie Rouland and
29
30 485 Joël Cuoq.
31
32 486 This study was partly supported by the European Community's 7th Framework Programme
33
34 487 (FP7/2007-2013) under grant agreement no. 278486 acronym "DEVELAGE" (EZ, JMV).
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3 618 **Figure 1. Setup of the manometrical investigation.**
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7 620 **Figure 2. Investigation of a 2 year old mouse lemur with the TonoVet®.**
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11 622 **Figure 3. Investigation of a 2 year old mouse lemur with the TonoPen™**
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15 624 **Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical**
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17 625 **control.** Manometrically determined IOP for the eight eyes of four animals are represented
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19 626 on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot
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21 627 represents the mean from three measurements per step/per eye. The dashed line represents the
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23 628 regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and
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25 629 TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.
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29 631 **Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in**
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31 632 **12 animals.** The results show high variation in measurements for the TonoPen™ and much
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33 633 more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right
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35 634 eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Tab.
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41 637 **Figure 6. Relation between tIOP values and age in healthy mouse lemur eyes.** This
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43 638 scatterplot shows the tIOP values for all measured healthy animals from Hannover and
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45 639 Montpellier ($N = 258$) on the y-axis in relation to age on the x-axis. The decrease in IOP is
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47 640 statistically not significant ($p = 0.077$, $r_s = -0.110$).
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Table 1. Overview of intraocular pressure (IOP) values in 12 animals for TonoVet® and TonoPen™.

The median, range, mean and standard deviation have been calculated for each instrument using the median from each animal's right and left eye.

Animal name	TonoVet® IOP results (mmHg)		TonoPen™ IOP results in mmHg	
	Right eye	Left eye	Right eye	Left eye
Pixel ♂ (2 years)	10	9	27	22
Quilla ♀ (3 years)	11	11	21	29
Quinn ♂ (3 years)	9	10	18	25
Quelle ♀ (3 years)	9	8	24	28
Quirl ♂ (3 years)	8	7	17	16
Pippi ♀ (2 years)	10	6	22	14
Paloma ♀ (2 years)	10	9	23	25
Peanut ♂ (2 years)	9	10	16	38
Quentin ♂ (3 years)	11	11	28	18
Queenie ♀ (3 years)	8	12	19	33
Pandora ♀ (2 years)	7	10	27	26
Phil ♂ (2 years)	7	9	31	25
Median (mmHg)	9		24.5	
Range (mmHg)	6-12		14-38	
Mean ± SD (mmHg)	9.2 ± 1.53		23.83 ± 5.89	

1 **Table 2. Variation of calibrated intraocular pressure (IOP) values in different species**

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Species	Reference	Mean IOP or range	Standard deviation
Human (N > 100.000)	[43]	15-16 mmHg	± 2.5-3 mmHg
Macaques (N= 102)	[44]	14.9 mmHg	± 2.1 mmHg
Gray mouse lemur (N = 516)	this study	20.3 mmHg	± 2.85 mmHg
Cats (N = 57)	[29]	20.74 mmHg	± 0.49 mmHg
Horses (N = 35)	[39]	22.1 mmHg	± 5.9 mmHg
Rabbits (N = 38)	[34]	15-23 mmHg	
Lewis rats (N = 115)	[32]	17.3 mmHg	± 5.25 mmHg
Dogs (N > 900)	[25]	19.0 mmHg	± 5.7 mmHg

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