Noninvasive Measurement of Rodent Intraocular Pressure with a Rebound Tonometer

Wan-Heng Wang, J. Cameron Millar, Iok-Hou Pang, Martin B. Wax, and Abbot F. Clark

PURPOSE. The present study evaluated the applicability of a rebound tonometer in measuring intraocular pressure (IOP) in rats and mice.

METHODS. The accuracy of the TonoLab rebound tonometer was determined in cannulated mouse and rat eyes. IOP was manipulated by changing reservoir height, and tonometer pressure readings were recorded by an independent observer. IOP values were recorded in conscious Wistar rats and in four different strains of mice. The effects of anesthesia on IOP were evaluated in two different strains of mice.

RESULTS. The IOP readings generated by the rebound tonometer correlated very well with the actual pressure in the eye. In rats, this linear correlation had a slope of 0.96 ± 0.05 (mean ± SEM, n = 4) and a Y-intercept of −2.1 ± 1.2. In mice, the slope was 0.99 ± 0.05 (n = 3), and the Y-intercept was 0.8 ± 1.4. Using this method, the resting IOP of conscious male Wistar rats was observed to be 18.4 ± 0.1 mm Hg (n = 152). In mice, strain differences in IOP were detected. Baseline IOP values in Balb/c, C57-BL/6, CBA, and 11- to 12-month-old DBA/2J mice were 10.6 ± 0.6, 13.3 ± 0.3, 16.4 ± 0.3, and 19.3 ± 0.4 mm Hg (n = 12), respectively. In separated studies, anesthesia lowered IOP from 14.3 ± 0.9 to 9.2 ± 0.5 mm Hg (n = 8) in C57-BL/6 mice, and from 16.6 ± 0.4 to 9.4 ± 0.6 mm Hg (n = 10) in CBA mice.

CONCLUSIONS. The rebound tonometer was easy to use and accurately measured IOP in rats and mice. This technique, together with advances in genetic and other biological studies in rodents, will be valuable in the further understanding of the etiology and pathology of glaucoma. (Invest Ophthalmol Vis Sci. 2005;46:4617–4621) DOI:10.1167/iovs.05-07851

Elevated intraocular pressure (IOP) is a major risk factor for glaucoma, which causes visual impairment and blindness in millions of patients each year. The cellular and molecular mechanisms involved in this disease remain elusive. A major obstacle for the advancement of understanding in the related biological processes is the lack of appropriate animal models. However, recent exciting developments in rodent glaucoma models provide promising avenues for future research in this field.

To take full advantage of the rodent models, it is necessary to assess IOP in rodents accurately and reproducibly. Unfortunately, because of its small size, the rodent eye presents a technical challenge for this task. Most tonometers designed for human or veterinarian uses are simply too large or cumbersome and therefore unsuitable for rats or mice. Consequently, an accurate and convenient method to measure rodent IOP has become an urgent need in glaucoma research.

In the past years, a number of methods have been used to measure IOP in rats and mice. Some, such as the microcanulation technique and the servo-null micropipette system, are invasive, puncturing the cornea. These methods cannot be used in conscious animals, or performed repeatedly in the same eye. Although currently available noninvasive methods overcome part or all of these disadvantages, many still have shortcomings that limit their popularity. For example, a Schiotz-like indentation method was successfully used to measure mouse IOP, but the animal needs to be anesthetized during the procedure. A modified Goldmann applanation tonometer has been reported to accurately measure IOP in conscious rats and mice. At the present time, the Tono-Pen applanation tonometer (Medtronic, Jacksonville, FL) appears to be the most popular noninvasive method for monitoring rat IOP, and it is also useful for measuring mouse IOP. Nonetheless, to obtain satisfactory results, extensive practice and careful attention to procedural details are required. Furthermore, since this instrument is designed for larger eyes, correction factors derived from empirically generated calibration curves are absolutely crucial for calculation of the actual rodent IOP.

Recently, an induction/impact tonometer was introduced. This equipment propels a lightweight magnetic probe to impact the cornea. The maximum deceleration of the probe during the impact correlates with IOP. Prototypes of this instrument were shown to produce meaningful data in human subjects and in rats. Lately, a handheld apparatus based on this technical principle became commercially available, marketed as the TonoLab rebound tonometer (Colonial Medical Supply, Franconia, NH). In the present study, we evaluated this tonometer for its accuracy, reproducibility, and applicability in measuring rat and mouse IOP.

MATERIALS AND METHODS

Animals

All animal procedures performed in this study complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 250 to 500 g and four strains of mice, Balb/c (1–4 months old), C57-BL/6 (1–4 months old), CBA (1–3 months old), and DBA/2J (11–12 months old) (Jackson Laboratories, Bar Harbor, ME), were used in this study. The animals were housed in transparent plastic rodent boxes under a 12-hour light-dark cycle with lights on starting at 6 AM. Food and water were available ad libitum.

IOP Measurement

IOP was measured using the TonoLab rebound tonometer for rodents (Colonial Medical Supply) according to the manufacturer’s recommended procedures. The tonometer was validated as described below before it was used to assess IOP in conscious animals. All IOP mea-
surements, except those related to tonometer validation, were performed between 10 AM and noon.

**Tonometer Validation**

To establish the correlation between the IOP values measured by the tonometer and the actual IOP, Wistar rats and Balb/c mice were anesthetized using a rat anesthesia solution (acepromazine [3 mg/kg; Vetus; Burns Veterinary Supply, Westbury, NY], ketamine [33 mg/kg; Fort Dodge, Madison, NJ], and xylazine [7 mg/kg; Vetus; Burns Veterinary Supply]; intramuscular injection) or a mouse anesthesia solution (acepromazine [1.8 mg/kg], ketamine [73 mg/kg], and xylazine [1.8 mg/kg]; intraperitoneal injection), respectively.

In rats, a 30-gauge needle was inserted through the cornea into the anterior chamber. The needle was attached via polyethylene tubing to a three-way connector, which in turn was connected in parallel to a pressure transducer (Model P23XL; Ohmeda, Singapore) and a fluid reservoir. The needle, tubing, connector, reservoir, and pressure transducer were filled with sterile intraocular irrigating solution (BSS Plus; Alcon Laboratories, Fort Worth, TX). One individual then randomly altered the height of the reservoir to change the hydrostatic pressure inside the eye, which was detected by the attached transducer and amplified, displayed, and recorded by a polygraph (Model 7D; Grass Instrument Company, Quincy, MA). A second individual, who was masked to the position of the reservoir and transducer readings, measured IOP with the rebound tonometer.

In mice, a similar validation procedure was used. However, because of the small volume of the anterior chamber, the 30-gauge needle was inserted instead through the sclera into the vitreous.

**IOP Measurement in Conscious Rats**

The rebound tonometer was fixed to a ring stand with clamps. Initially, during the training session, rats were slightly sedated by an intramuscular injection of acepromazine (2 mg/kg). Approximately 5 minutes later, they were placed on a platform of adjustable height and gently restrained by hand with the eye adjacent to the tonometer tip (Fig. 1). Tonometer IOP readings of both eyes were assessed. After several sessions, however, the animals became acclimated to the procedure and no sedative was needed. Once the animals were acclimated, the whole procedure took 1 to 2 minutes and was well tolerated; the animals generally remained calm during the measurement.

**IOP Measurement in Conscious Mice**

Mice were gently restrained by first being placed in a soft, clear plastic cone (Decapicone; Braintree Scientific, Inc., Braintree, MA), then secured in a custom-made restrainer. The latter was then placed on a platform of adjustable height (Fig. 2). After a few minutes of acclimation, the mice usually appeared calm and comfortable. IOP measurements were then performed using the rebound tonometer.

**IOP Measurement in Anesthetized Mice**

Mice were anesthetized as described above. Sufficient anesthesia was confirmed by the loss of stable sternal recumbency, which occurred typically 3 to 7 minutes after injection of the anesthetic agents. After an additional 5 minutes, IOP was measured with the rebound tonometer.

**Data Analysis**

Data are reported as means ± SEM. Two-tailed Student’s t-test was used to compare between two groups of results, and one-way ANOVA followed by Bonferroni’s multiple comparison was used to compare among three or more groups. Differences were regarded as significant when $P < 0.05$.

**RESULTS**

**Tonometer Validation**

The TonoLab rebound tonometer generated IOP readings that correlated very well with the IOP measured by the pressure transducer. Figure 3 shows example results of validation studies in the rat and mouse models. The correlation was reproducible in several independent studies, each with a regression coefficient ($r^2$) larger than 0.94. In the rat model, the mean slope of the four individual regression lines relating the measured and the actual IOP was 0.96 ± 0.05 ($n = 4$), and the mean $Y$-intercept was $-2.1 ± 1.2$. In the mouse model, the
regression lines had a mean slope of 0.99 \( \pm 0.05 \) \((n = 3)\), and a mean Y-intercept of 0.8 \( \pm 1.4\). The mean slope derived from either the rat or mouse model was not significantly different from 1 and the Y-intercept not significantly different from 0.

### IOP of Conscious Animals

Using the rebound tonometer, the resting IOP of conscious and unsedated male Wistar rats measured between 10 AM and noon was 18.4 \( \pm 0.1 \) mm Hg \((n = 132)\). This value is similar to that previously reported.\(^9\)

In mice, it was demonstrated earlier that different strains have different baseline IOP values.\(^{14}\) We observed similar diversity among the mouse strains with the rebound tonometer. Thus, in conscious Balb/c mice, an IOP of 10.6 \( \pm 0.6 \) mm Hg \((n = 12)\) was detected. Higher IOP values were recorded in the C57-BL/6 and CBA mice \((13.3 \pm 0.3\) and 16.4 \( \pm 0.3 \) mm Hg, respectively\). Eleven- to 12-month-old DBA/2J glaucomatous mice had an elevated IOP of 19.3 \( \pm 0.4 \) mm Hg \((\text{Fig. 4})\). These IOP differences between strains were significant \((P < 0.05\) by one-way ANOVA with Bonferroni’s multiple comparison).

IOP measurement using the rebound tonometer appeared to be well tolerated by both rats and mice. The animals exhibited no signs of irritation or discomfort during the procedure. At various time points after even repeated daily IOP measurements for up to a month, ophthalmoscopic examination revealed no corneal abnormality, ocular hyperemia, or symptoms of inflammation \((\text{data not shown})\). The animals were judged normal in their overall appearance and behavior by staff veterinarians.

### IOP of Anesthetized Mice

Anesthesia is known to affect IOP.\(^{15}\) This change was also detected with the rebound tonometer. As shown in Figure 5, IOP was significantly reduced in anesthetized C57-BL/6 and CBA mice. In C57-BL/6 mice, anesthesia lowered the IOP of conscious animals from 14.3 \( \pm 0.9\) to 9.2 \( \pm 0.5 \) mm Hg \((n = 8)\). In CBA mice, the IOP of conscious animals decreased from 16.6 \( \pm 0.4\) to 9.4 \( \pm 0.6 \) mm Hg \((n = 10)\).

### DISCUSSION

In the current study, we demonstrated that the TonoLab rebound tonometer can monitor rat and mouse IOP accurately and reproducibly. In the validation studies, the measured IOP values were similar to the actual pressure within the eye. Using procedures recommended by the manufacturer, no correction factors are needed to calculate the actual IOP. The instrument uses a minimal force to propel a very lightweight probe, whose impact with the cornea is almost imperceptible by human subjects, such that local corneal anesthesia is not necessary.\(^{12}\)

In rodents, we also found that the animals appeared to tolerate the procedure very well in the absence of any corneal anesthetic agent. There were no observable ocular or systemic untoward effects either during the IOP measurement or at various time points afterward. The IOP values of conscious Wistar rats and various strains of mice recorded by the rebound tonometer were consistent with those reported using other techniques.\(^9,10,14\) The reduction in IOP induced by general anesthesia described previously\(^{15}\) was also easily identified by the rebound tonometer. We further confirmed mouse-strain

---

![Figure 3](https://example.com/figure3.png)  
**Figure 3.** Examples of correlation between the measured IOP and the actual IOP in the rat (upper) and mouse models (lower). TonoLab tonometer IOP measurements were obtained by an operator who was unaware of the actual IOP. Ten measurements were obtained from each preset actual IOP. Values are means \( \pm \) SEM of 10 readings. Additional independent studies (rat model, \(n = 4\); mouse model, \(n = 5\)) generated similar correlations.

![Figure 4](https://example.com/figure4.png)  
**Figure 4.** Difference in resting IOP in different mouse strains. Each symbol represents the mean of 10 to 15 measurements from each mouse eye. Differences between any two groups were significant, \(P < 0.05\); one-way ANOVA with Bonferroni’s multiple comparison.
differences in IOP that closely matched previously reported literature values. Compared with other techniques, use of the rebound tonometer was much easier to learn and did not require lengthy practice before satisfactory data were obtained. In addition, because of the very light force used by the tonometer when impacting the cornea, the rat or mouse IOP appeared not to be affected by multiple repeated measurements. There was no hint of IOP decline even after up to 90 minutes. There was no indication that the tonometer probe would produce an artificial change in IOP. As indicated by the results, this restraining device worked well, and assessment of mouse IOP could be easily achieved by a single operator.

In addition, we found that IOP values obtained by the rebound tonometer were very sensitive to various factors. For example, the IOP of an even slightly agitated animal will be significantly higher than normal. Consequently, we recommend multiple practice sessions to familiarize the animals with the handling and measurement procedure so as to minimize their excitement. A quiet and serene environment in the laboratory where IOP is studied is also crucial. Disturbances in the surroundings tend to upset the animals and cause erratic IOP readings. Most important, for accurate IOP measurement, it is highly critical to aim the contact point of the tonometer as close to the apex of the cornea as possible and to carefully align the tonometer tip with the optical axis of the eye. Misalignment does not necessarily trigger an error message from the equipment, but it often caused the reporting of a lower IOP reading than the actual value. This error in underreporting is especially obvious in mouse eyes with higher IOP, much more so than in normotensive eyes. Thus, misalignment of the tonometer probe will generate false-negative data in ocular hypertensive treatments and false positive results in hypotensive studies. Consequently, vigilant care and meticulous attention to the placement and alignment of the tonometer are essential to avoid this drawback. With these precautions, reproducible and meaningful IOP values can be routinely generated with the rebound tonometer.

Recently, many rodent glaucoma models have been developed and characterized. In rats, ocular hypertension can be induced by surgical procedures that damage the aqueous outflow pathway, such as by injecting hypertonic saline into one of the episcleral veins, by laser destroying the trabecular meshwork and/or the episcleral vessels, or by cauterizing the extraocular veins. Moreover, an increasing number of mutant mice and rats that spontaneously develop glaucoma have been discovered in the past years. For example, the DBA/2 mouse strain exhibits symptoms of pigmentary glaucoma due to iris atrophy and iris pigment dispersion. A similar but not identical strain, the DBA/2N mouse, also shows ocular hypertension with analogous etiology.

The AXKD-28/Ty mouse shares the iris stromal atrophy phenotype and glaucoma. The Coll1a1(rr) mutant mouse develops open-angle glaucoma associated with an impaired degradation of type I collagen, an extracellular matrix protein, in the trabecular meshwork. Finally, a newly described rat strain acquires increased IOP associated with a ciliary body hypertrophy. These rodent glaucoma models did and will continue to contribute to our increasing understanding of the mechanisms involved in the development of the disease. An improved capability of expedient and precise assessment of rat and mouse IOP will allow us to take full advantage of these experimental models.

Furthermore, applications of molecular biological techniques and genetic manipulation in rodents have also been instrumental in furthering our knowledge of the etiology and pathology of glaucoma. The discovery of glaucoma genes coupled with the ever-expanding capacity in manipulating these genes in vivo in rodents by enhancing or suppressing gene expression will help in identifying the critical glaucomatous molecular and cellular pathways involved for each glaucoma gene, as well in ascertainment of the final common pathways of the disease. The availability of a convenient, re-
produced, and accurate rodent IOP assessment will be extremely helpful in determining the role of elevated IOP in glaucoma pathogenesis.

References