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Circadian rhythm of intraocular pressure in the adult rat

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Abstract

Ocular hypertension is a risk factor for developing glaucoma, which consists of a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells and subsequent irreversible vision loss. Our understanding of how intraocular pressure damages the optic nerve is based on clinical measures of intraocular pressure that only gives a partial view of the dynamic pressure load inside the eye. Intraocular pressure varies over the course of the day and the oscillator regulating these daily changes has not yet been conclusively identified. The purpose of this study was to compare and contrast the circadian rhythms of intraocular pressure and body temperature in Brown Norway rats when these animals are housed in standard light-dark and continuous dim light (40–90 lux) conditions. The results from this study show that the temperature rhythm measured in continuous dim light drifted forward relative to external time, indicating that the rhythm was free running and being regulated by an internal biological clock. Also, the results show that there is a persistent, but dampened, circadian rhythm of intraocular pressure in continuous dim light and that the circadian rhythms of temperature and intraocular pressure are not synchronized by the same central oscillator. We conclude that once- or twice-daily clinical measures of intraocular pressure are insufficient to describe intraocular pressure dynamics. Similarly, our results indicate that, in experimental animal models of glaucoma, the common practice of housing animals in constant light does not necessarily eliminate the potential influence of intraocular pressure rhythms on the progression of nerve damage. Future studies should aim to determine whether an oscillator within the eye regulates the rhythm of intraocular pressure and to better characterize the impact of glaucoma on this rhythm.

Keywords

Circadian; core body temperature; intraocular pressure; rodent; telemetry

INTRODUCTION

Glaucoma is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells and their axons that ultimately leads to irreversible vision loss.

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DECLARATION OF INTEREST

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Elevated intraocular pressure is a risk factor for developing glaucoma and several studies have indicated that lowering intraocular pressure slows progression (AGIS, 2000; Francis et al., 2008; Heijl et al., 2002; Kass et al., 2002; Nickells et al., 2012; Sommer et al., 1991; Weinreb et al., 2014). Much of what is known about glaucoma has come from animal models, including those involving rats (Morrison et al., 1997; Samsel et al., 2011; Shareef et al., 1995; Ueda et al., 1998; Yu et al., 2006), that have characterized the structural and functional changes associated with elevated intraocular pressure (Frishman et al., 2000; Harwerth et al., 2002, 2010; Vidal-Sanz et al., 2012). However, there remains ongoing debate about the influence of daily variations in intraocular pressure on the development and progression of glaucoma.

Our understanding of intraocular pressure dynamics and its contribution to the disease process is partly limited by the tonometry techniques commonly employed in clinical practice. Usually, a single intraocular pressure measurement is taken at each patient visit, which often occurs months apart. These intraocular pressure “snapshots” are insufficient to characterize pressure variations due to disease, in addition to typical daily pressure variations. A circadian rhythm of intraocular pressure is known to exist in normal human eyes (David et al., 1992; Liu et al., 1998, 1999, 2003), rodents (Aihara et al., 2003; Li & Liu, 2008; Mermoud et al., 1994; Moore et al., 1996), rabbits (Rowland et al., 1981), cats (Del Sole et al., 2007) and dogs (Piccione et al., 2010). These circadian fluctuations in intraocular pressure might be associated with disease progression among glaucoma patients (Caprioli & Coleman, 2008; Musch et al., 2011). Quantifying the circadian rhythm of intraocular pressure may therefore help identify which aspect of intraocular pressure is most significantly correlated with disease and its progression (e.g. between-visit fluctuations, peak intraocular pressure, daily fluctuations, etc.) and this could lead to more effective glaucoma treatment strategies.

While a circadian rhythm of intraocular pressure has been measured under normal environmental conditions (e.g. regular day-night cycles), the rhythm was not detected in rats (Morrison et al., 2005), rabbits (Rowland et al., 1981) and dogs (Piccione et al., 2010) kept in a continuous light environment. However, these studies did not simultaneously monitor the animals' circadian phase, which is known to drift in constant light or dark environmental conditions that enable an animal's internal circadian clock to “free-run”. Without an external time cue (light onset or offset) to synchronize the intraocular pressure rhythms, inter-individual variations in the free-running circadian period would cause the pressure rhythms for rats in an experimental cohort to go out of phase with each other. By grouping the intraocular pressure measurements by an external clock, the presence of any persistent cyclical rhythm could be masked or appear considerably dampened. The purpose of this study was to measure the circadian rhythm of intraocular pressure in brown Norway rats and determine whether a central oscillator regulates this rhythm. To test this hypothesis, we compared IOP rhythms occurring under constant light conditions to core body temperature rhythms, the latter serving as an independent marker of circadian phase.

METHODS

The Institutional Animal Care and Use Committee of the University of Houston approved all experimental procedures. Animal care procedures and experiments were carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and conform to international ethical standards (Portaluppi et al., 2010). Eleven adult male brown Norway rats (average $\pm$ SD weight: 357 ± 32 g) were housed individually in transparent cages with food and water available *ad libitum* and were replenished every 3 to 4 days.

The circadian rhythms of core body temperature and intraocular pressure were assessed in two groups of animals. Figure 1 illustrates the experimental timeline. Briefly, temperature was measured in Group 1 ($n=4$ animals) in standard light-dark environmental conditions (LD) and in continuous dim light (40–90 lux) for four weeks. In Group 2 ($n=7$ animals), temperature was recorded in continuous dim light (40–90 lux) for 7 weeks and then for 1 month during re-entrainment to standard light-dark environmental conditions. The circadian rhythm of intraocular pressure was characterized by measuring intraocular pressure every two hours over the course of 26 h. Three 26-hour experiments were performed in Group 1; one in standard light-dark environmental conditions and two in continuous dim light (after 1 and 4 weeks exposure to continuous dim light). Two 26-hour experiments were carried out with Group 2; one after 7-weeks of continuous dim light exposure and another one after the animals re-entrained to standard light-dark environmental conditions. These experiments are further discussed in the following methods sections.

Quantifying the circadian rhythm of core body temperature

Core body temperature was measured with a temperature sensor that was implanted intraperitoneally (Respironics Company, Mini Mitter, Bend, OR). The implant sensor was set to automatically record core body temperature every 5 min for five weeks in Group 1 and for 11 weeks in Group 2. Animals were kept in standard light-dark environmental condition (lights on at 8 AM and lights off at 8 PM) prior to implanting the temperature sensor. Rats were anesthetized during the implant surgical procedure with an intraperitoneal injection of ketamine (Vedco, St. Joseph, MO; 50 mg/kg) and xylazine (Vedco; 5 mg/kg). Post-operative pain was minimized with a single dose of ketoprofen (Fort Dodge Animal Health, Fort Dodge, IA; 100 mg/mL). Animals were allowed to recover from surgery for one week before temperature data was included in the study (Figure 1).

The circadian rhythm of core body temperature was evaluated by fitting a cosine function to the raw daily temperature data (Cornelissen, 2014; Halberg et al., 1967; Refinetti et al., 2007). This procedure is commonly referred to as a cosinor analysis. Various parameters were extracted from the cosinor analysis, including the daily mean core body temperature (M ; Equation (1)), the time when the maximum core body temperature occurs (ϕ ; Equation (1)), and the maximum range of temperature measurements within a day ($2 \times A$; Equation (1)). Temperature onset time, defined as the time when temperature started to rise above the 24-hour mean, was calculated by taking the first derivative of the cosine fit function. Temperature period length (τ) was calculated from the linear regression slope of daily temperature onset times versus all days that temperature was recorded. The existence of a

rhythm was quantified by calculating daily rhythmicity (correlation of determination of the cosine fit multiplied by 100%) and by testing the null hypothesis of zero-amplitude at a statistically significant level of probability (F -test; $p < 0.05$).

$$y = M + A \cos \left(\frac{2\pi}{\tau} \times t + \varphi \right) \quad (1)$$

Quantifying the circadian rhythm of intraocular pressure

Intraocular pressure was measured in both eyes from conscious rats every two hours over a 26-hour period (Figure 1). Prior to surgical implantation of the temperature sensors, animals were trained to hold still during intraocular pressure measurements. This behavioral training was performed over the course of one week prior to surgery. During the 26-hour experiments, two-trained handlers measured intraocular pressure using calibrated rebound tonometers (TonoLab, Icare, Vantaa, Finland; see: Danias et al., 2003; Kontiola et al., 2001) at each time point. During rebound tonometry, a small, magnetic, plastic-tipped pin is propelled towards the corneal apex. Intraocular pressure is proportional to the pin's rebound speed. Ten pressure measurements were acquired for each eye every two hours, for the full 26-hour measurement period. A deep-red headlight was used to facilitate the measurement of intraocular pressure during the dark phase of the light-dark environmental condition. To minimize systematic bias, the order in which animal was measured, the tonometer used to acquire the measurements, and the investigator handling each animal were randomized. Intraocular pressure measurements from only the right eyes were included in the analysis. The circadian rhythm of intraocular pressure was quantified using the previously described cosinor analysis. The difference was that the period length (τ) of the cosine fit (Equation (1)) was set to the temperature period length so that the phase relationship of the intraocular pressure rhythms could be quantified in relation to the animal's core body temperature rhythm.

Phase relationship between core body temperature and intraocular pressure

The phase relationship between core body temperature and intraocular pressure was investigated to evaluate if these two physiological functions are synchronized to the same central oscillator. This phase relationship was plotted in polar graphs to visually inspect the concordance between temperature and intraocular pressure phase. For example, the clustering of all points in the same polar quadrants supports the premise that the same central oscillator regulates both physiological functions. The polar graphs were generated by calculating the time at which the maximum temperature and intraocular pressure occurred. These times were then transformed from peripheral clock time to either Zeitgeber Time (for standard light-dark environmental conditions) or Circadian Time (for continuous dim light). In Zeitgeber Time, light onset is recorded as the 0.0 h timepoint and light offset is recorded as the 12.0 h timepoint in a standard 12 h/12 h light/dark cycle. Circadian Time is based on the free-running period of a rhythm and the temperature onset time (defined above) in nocturnal animals is set as the 12.0 h timepoint. Time in the polar graphs was indicated by the angle of a vector whose length was 1 when analyzing the temperature and intraocular pressure data measured in standard light-dark environmental condition. In continuous dim

light, the length of the vector was set to a ratio that was normalized to values measured in standard light-dark conditions. This ratio was used to highlight if the rhythm dampened in continuous dim light. Lastly, the phase relationship was quantified by measuring the difference between the time when core body temperature and intraocular pressure were at their maximum. Circular statistics (e.g. length of the mean resultant vector, circular variance and Rayleigh's test) were used to determine the distribution of temperature and IOP peak times (Berens, 2009). For example, a mean resultant vector of one and circular variance of zero would suggest that, for all animals, intraocular pressure was at a maximum at the same time. Rayleigh's test yields 1 if peak times are uniformly distributed around the clock.

RESULTS

Quantifying the circadian rhythm of core body temperature

Core body temperature in standard light-dark environmental conditions was lowest during the light-phase (36.9 ± 0.1 °C; 95% CI: 36.8 °C–37.0 °C), highest during the dark-phase (37.5 ± 0.2 °C; 95% CI: 37.4 °C–37.7 °C), and peaked near the middle of the dark phase (17.5 ± 1.9 Zeitgeber Time; 95% CI: 17.1 to 17.8 Zeitgeber Time). Other parameters of the body temperature rhythm are summarized in Table 1. A persistent but dampened temperature rhythm was measured in continuous dim light ($p < 0.001$). For example, the maximum range in temperature measurements within a day was larger in standard light-dark environmental conditions (0.60 ± 0.11 °C) than that measured after 7 weeks of exposure to continuous dim light (0.34 ± 0.07 °C).

Figure 2 shows temperature data recorded from one animal and illustrates the circadian rhythm of temperature that occurs under continuous dim light (Figure 2A) and standard 12 h/12 h light-dark environmental conditions (Figure 2B–C). In continuous dim light, the temperature rhythm drifted forward relative to external time, indicating that the rhythm was free-running and being regulated by an internal biological clock (Figure 2A). Temperature rhythm drifted forward at a rate that ranged between 0.5 to 0.8 hours per day, which translated to a longer period length (24.6 ± 0.3 h; 95% CI: 24.5–24.8 hours) than that measured in light-dark conditions ($p = 0.001$). This forward drift persisted for four days after the animal returned to standard light-dark conditions before synchronizing with the external lighting cycle once again (Figure 2B). Temperature period length in the light-dark condition was 24.0 ± 0.2 h (95% CI: 23.9–24.2 h; Figure 2C).

Quantifying the circadian rhythm of intraocular pressure

Intraocular pressure measurements acquired from two animals during the 26-hour experiments are shown in Figure 3. Intraocular pressure in the standard light-dark condition was lowest during the light-phase (16 ± 2 mmHg), highest during the dark-phase (30 ± 7 mmHg), and peaked near the middle of the dark phase (16.6 ± 1.2 Zeitgeber Time; 95% CI: 15.8 to 17.4 Zeitgeber Time). Rhythmicity analysis showed that a dampened circadian rhythm of intraocular pressure persisted while the animals were maintained in continuous dim light ($p < 0.001$; Table 2). The maximum range of intraocular pressure measurements was 14 ± 3 mmHg under light-dark conditions, and this maximum range dampened to 8 ± 1

mmHg after 1 week (Group 1), 8 ± 2 mmHg after 4 weeks (Group 1) and 6 ± 1 mmHg after 7 weeks (Group 2) of exposure to continuous dim light (Table 2).

Phase relationship between core body temperature and intraocular pressure

The relationship between core body temperature and intraocular pressure are illustrated in Figure 4 (for all Group 1 animals) and Figure 5 (for all Group 2 animals). Temperature and intraocular pressure peaked at a similar time when the animals were housed in the standard 12 h/12 h light-dark conditions. The mean difference between the timepoints for maximum temperature and intraocular pressure measurements was not significantly different from zero for Group 1 (1.8 ± 1.4 h; 95% CI: -0.4 to 4.1 h; $p=0.08$) or for Group 2 (-0.1 ± 0.6 h; 95% CI: -0.63 to 0.43 h; $p=0.65$), indicating that both physiological functions peaked at the same time under these environmental conditions. However, temperature and intraocular pressure peaked at different times when the animals were placed in continuous dim light. In Group 1, this time difference was -4.6 ± 1.0 h (95% CI: -6.1 to -3.0 h) after 1 week of continuous dim light exposure and 9.5 ± 6.8 h (95% CI: -1.3 to 20.3 h) after 4 weeks of continuous dim light exposure. In Group 2, the time difference was 6.2 ± 8.4 h (95% CI: -1.5 to 14.0 h) after 7 weeks of continuous dim light exposure. These results are further summarized in the polar graphs shown in Figure 6 and Table 2. The polar graphs in Figure 6 show that, in standard light-dark environmental conditions, both maximum temperature and maximum intraocular pressure occurred near the middle of the dark phase (Figure 6A). Furthermore, Tables 1 and 2 show that the resultant vector for temperature and IOP were larger than 0.96, circular variances were smaller than 0.04, and Rayleigh's test ($p<0.001$) supported that the times were concentrated around the same time (e.g. the middle of the dark-phase). In continuous dim light, Circadian Time is based on the free-running period of a rhythm and the temperature onset time in nocturnal animals is set as the 12.0 h time point. For this reason, the temperature peak times for all animals line up at the same time (Figure 6B–D). These figures also show that, in continuous dim light, the time when intraocular pressure peaked drifted further away from the time when temperature peaked (Figure 6B–D). When intraocular pressure peaked, it was normally distributed around the clock after 7 weeks of exposure to continuous dim light (Figure 6D). These results are further explained by the circular statistics shown in Table 2, where the resultant vector for IOP was 0.98 after 1 week in continuous dim light and decreased to 0.11 after 7 weeks in continuous dim light. Also, the circular variance increased from 0.02 after 1 week in continuous dim light to 0.89 after 7 weeks in continuous dim light. Lastly, the p values from Rayleigh's test were larger than 0.85, suggesting that IOP peak times were uniformly distributed around the clock (Figure 6D). These results collectively indicate that the circadian phase between core body temperature and intraocular pressure was synchronized under light-dark environmental conditions, but then the circadian phase of intraocular pressure rhythm drifted relative to the phase of core body temperature rhythm under constant light conditions.

DISCUSSION

In this study, we quantified the circadian rhythm of intraocular pressure in adult rats and determine the phase relationship between the timing for this rhythm and that for core body temperature, a commonly used circadian phase marker.

A normal temperature circadian rhythm was measured under standard light-dark environmental conditions and a dampened but significant rhythm was still present after up to seven weeks of continuous dim light exposure. It has been previously reported that, although there is a gradual decrease in the amplitude, circadian temperature rhythms can still be observed in rats that have been maintained in continuous light conditions for several weeks (Depres-Brummer et al., 1995; Eastman & Rechtschaffen, 1983; Honma & Hiroshige, 1978). Light intensity is also an important consideration when measuring any physiological function under free-running conditions because the coherence of the rhythm decreases more quickly with increasing light intensity (Carpenter & Grossberg, 1984; Rosenwasser, 1993). With the dim light used in the current study (40–90 lux), our finding that the temperature rhythms persisted after one to seven weeks in constant light conditions is therefore consistent with the results reported in these previous studies.

Others have shown the TonoLab rebound tonometer to be a repeatable and accurate method of measuring intraocular pressure in rats (Morrison et al., 2009; Wang et al., 2005), and we have verified the applicability of this device in our lab (Lozano DC et al., IOVS, 2009, 50: ARVO E-Abstract: 2853). With this tonometer, we quantified the fluctuation in intraocular pressure that occurs across a standard 12 h/12 h light-dark cycle, and showed that the peak of this pressure rhythm occurs during the dark phase, as has been described previously (Moore et al., 1996). In the current study, a smaller-amplitude but significant intraocular pressure rhythm could still be detected after the animals had been exposed to continuous dim light for one to seven weeks (Table 2). Morrison and colleagues reported that no circadian rhythm of intraocular pressure was detected in brown Norway rats housed in continuous dim light for 35 days, even though their experimental set-up and light intensity was similar to that used in the current study (Morrison et al., 2005). However, in this prior study, intraocular pressure was only measured twice a day and the timing of these measurements was based on an external (morning and evening) timing schedule. In nocturnal animals exposed to continuous light, the circadian period generally lengthens (Aschoff, 1960), a finding that is consistent with the temperature rhythm data outlined in the present study. Therefore, the peak and trough for daily IOP values would be expected to gradually shift, in terms of external time, from when they occurred while the animals were maintained on a standard light-dark condition. Furthermore, due to the absence of any external synchronization cues (i.e. zeitgebers) during the continuous light exposure, variations in each animal's circadian period would cause the animals in an experimental cohort to gradually become significantly out-of-phase with each other over time. Averaging data that was obtained from multiple animals at potentially different times in their internal circadian phase would mask detection of an underlying rhythm. In this study, we obtained 13 intraocular pressure measurements over a 26-hour period, and performed cosinor analysis on data obtained from each individual animal. This may explain the apparent discrepancy in our finding of a significant rhythm for intraocular pressure that persisted for weeks in constant light, as opposed to those reported in previous studies on rats, rabbits and beagles that characterized rhythmicity using time-point averaged data (Morrison et al., 2005; Piccione et al., 2010; Rowland et al., 1981).

Comparing the rhythm of intraocular pressure to that for core body temperature, which served as an independent marker of circadian phase, we showed that these two rhythms

became uncoupled from each other after the animals had been exposed to the continuous light conditions (Figure 6). In the rat, the rhythms of core body temperature, locomotor activity and plasma corticosterone all remain synchronized with each other in continuous light conditions, indicating that these rhythms are regulated by a common central oscillator (Honma & Hiroshige, 1978). Our finding that the separation between the peak times for intraocular pressure and body temperature increased with longer exposure to continuous dim light suggests that a separate oscillator regulates the intraocular pressure rhythm. The location of this oscillator is a subject for future research, but one intriguing possibility is that it may be located peripherally within the eye itself.

Even though we have the ability to accurately and repeatedly measure intraocular pressure, this study still has the limitation that we only acquired a limited number of intraocular pressure measurements. These discrete measurements only give a partial view of intraocular pressure dynamics over the course of the day. New technology is now emerging to continuously monitor daily variations in intraocular pressure. For example, contact lenses (De Smedt et al., 2012; Lorenz et al., 2013; Mansouri et al., 2012; Twa et al., 2010) and implantable sensors (Melki et al., 2014) are available to measure intraocular pressure in humans. Telemetric systems are being developed and tested to acquire continuous intraocular pressure measurements in non-human primates (Downs et al., 2011), rabbits (McLaren et al., 1996; Schnell et al., 1996; Todani et al., 2011), rats (Valderrama et al., 2008) and mice (Li & Liu, 2008). A more thorough description of intraocular pressure dynamics will become possible and further enhance our understanding of how fluctuations in this physiological variable contribute to glaucomatous disease progression.

Rat models of elevated intraocular pressure have been used extensively to model glaucomatous pathophysiology. It has become standard practice to house the rats used in these experiments in constant light (Ahmed et al., 2004; Hartwick et al., 2005; Jia et al., 2000; Nissirios et al., 2008; Pang et al., 2005; Sposato et al., 2009). With the assumption that there is negligible daily variation in intraocular pressure under these conditions, the advantage of this approach is that a single measurement was thought to provide a reasonable estimate of the cumulative pressure exposure for that day. However, we found that a significant pressure rhythm can persist for weeks in constant light (see Figures 4 and 5 and Table 2). Thus, the daily variation in intraocular pressure may influence the progression of optic nerve damage in these experimental glaucoma models to a greater extent than previously appreciated. This finding supports the use of more continuous recording techniques in order to more definitively measure cumulative pressure exposure.

In summary, the results from this study show that there is a persistent circadian rhythm of intraocular pressure in continuous dim light and that the same central oscillator does not regulate the circadian rhythms of temperature and intraocular pressure similarly. Future studies on the rhythm of intraocular pressure should benefit from the development of more continuous data acquisition techniques, which will enable the impact of glaucoma on this rhythm to be better characterized.

References

- AGIS . The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. The AGIS Investigators. *Am J Ophthalmol.* 2000; 130:429–40. [PubMed: 11024415]
- Ahmed F, Brown KM, Stephan DA, et al. Microarray analysis of changes in mRNA levels in the rat retina after experimental elevation of intraocular pressure. *Invest Ophthalmol Vis Sci.* 2004; 45: 1247–58. [PubMed: 15037594]
- Aihara M, Lindsey JD, Weinreb RN. Twenty-four-hour pattern of mouse intraocular pressure. *Exp Eye Res.* 2003; 77: 681–6. [PubMed: 14609556]
- Aschoff J. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb Symp Quant Biol.* 1960; 25: 11–28. [PubMed: 13684695]
- Berens P. CircStat: A Matlab toolbox for circular statistics. *J Stat Softw.* 2009; 31:1–21.
- Caprioli J, Coleman AL. Intraocular pressure fluctuation a risk factor for visual field progression at low intraocular pressures in the advanced glaucoma intervention study. *Ophthalmology.* 2008; 115:1123–9. [PubMed: 18082889]
- Carpenter GA, Grossberg S. A neural theory of circadian rhythms: Aschoff's rule in diurnal and nocturnal mammals. *Am J Physiol.* 1984; 247:R1067–82. [PubMed: 6542316]
- Cornelissen G. Cosinor-based rhythmometry. *Theor Biol Med Model.* 2014; 11:16. [PubMed: 24725531]
- Danias J, Kontiola AI, Filippopoulos T, Mittag T. Method for the noninvasive measurement of intraocular pressure in mice. *Invest Ophthalmol Vis Sci.* 2003; 44:1138–41. [PubMed: 12601041]
- David R, Zangwill L, Briscoe D, et al. Diurnal intraocular pressure variations: an analysis of 690 diurnal curves. *Br J Ophthalmol.* 1992; 76:280–3. [PubMed: 1356429]
- De Smedt S, Mermoud A, Schnyder C. 24-Hour intraocular pressure fluctuation monitoring using an ocular telemetry sensor: Tolerability and functionality in healthy subjects. *J Glaucoma.* 2012; 21:539–44. [PubMed: 21602707]
- Del Sole MJ, Sande PH, Bernades JM, et al. Circadian rhythm of intraocular pressure in cats. *Vet Ophthalmol.* 2007; 10: 155–61. [PubMed: 17445076]
- Depres-Brummer P, Levi F, Metzger G, Touitou Y. Light-induced suppression of the rat circadian system. *Am J Physiol.* 1995; 268:R1111–16. [PubMed: 7771569]
- Downs JC, Burgoyne CF, Seigfreid WP, et al. 24-hour IOP telemetry in the nonhuman primate: Implant system performance and initial characterization of IOP at multiple timescales. *Invest Ophthalmol Vis Sci.* 2011; 52:7365–75. [PubMed: 21791586]
- Eastman C, Rechtschaffen A. Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiol Behav.* 1983; 31:417–27. [PubMed: 6657763]
- Francis BA, Varma R, Chopra V, et al. Los Angeles Latino Eye Study . Intraocular pressure, central corneal thickness, and prevalence of open-angle glaucoma: The Los Angeles Latino Eye Study. *Am J Ophthalmol.* 2008; 146:741–6. [PubMed: 18672218]
- Frishman LJ, Saszik S, Harwerth RS, et al. Effects of experimental glaucoma in macaques on the multifocal ERG. Multifocal ERG in laser-induced glaucoma. *Doc Ophthalmol.* 2000; 100:231–51. [PubMed: 11142748]
- Halberg, F.; Tong, YL.; Johnson, EA. Circadian system phase, an aspect of temporal morphology: Procedures and illustrative examples. In: von Mayersbach, H., editor. *The cellular aspects of biorhythms.* Berlin: Springer; 1967. p. 20–48.
- Hartwick AT, Zhang X, Chauhan BC, Baldrige WH. Functional assessment of glutamate clearance mechanisms in a chronic rat glaucoma model using retinal ganglion cell calcium imaging. *J Neurochem.* 2005; 94:794–807. [PubMed: 16001970]
- Harwerth RS, Crawford ML, Frishman LJ, et al. Visual field defects and neural losses from experimental glaucoma. *Prog Retin Eye Res.* 2002; 21:91–125. [PubMed: 11906813]
- Harwerth RS, Wheat JL, Fredette MJ, Anderson DR. Linking structure and function in glaucoma. *Prog Retin Eye Res.* 2010; 29: 249–71. [PubMed: 20226873]

- Heijl A, Leske MC, Bengtsson B, et al. Early Manifest Glaucoma Trial . Reduction of intraocular pressure and glaucoma progression: Results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol*. 2002; 120:1268–79. [PubMed: 12365904]
- Honma KI, Hiroshige T. Endogenous ultradian rhythms in rats exposed to prolonged continuous light. *Am J Physiol*. 1978; 235: R250–6. [PubMed: 727287]
- Jia L, Cepurna WO, Johnson EC, Morrison JC. Effect of general anesthetics on IOP in rats with experimental aqueous outflow obstruction. *Invest Ophthalmol Vis Sci*. 2000; 41: 3415–19. [PubMed: 11006233]
- Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: A randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002; 120:701–13. discussion 829–30. [PubMed: 12049574]
- Kontiola AI, Goldblum D, Mittag T, Danias J. The induction/ impact tonometer: A new instrument to measure intraocular pressure in the rat. *Exp Eye Res*. 2001; 73:781–5. [PubMed: 11846509]
- Li R, Liu JH. Telemetric monitoring of 24 h intraocular pressure in conscious and freely moving C57BL/6J and CBA/ CaJ mice. *Mol Vis*. 2008; 14:745–9. [PubMed: 18431454]
- Liu JH, Kripke DF, Hoffman RE, et al. Nocturnal elevation of intraocular pressure in young adults. *Invest Ophthalmol Vis Sci*. 1998; 39:2707–12. [PubMed: 9856781]
- Liu JH, Kripke DF, Twa MD, et al. Twenty-four-hour pattern of intraocular pressure in the aging population. *Invest Ophthalmol Vis Sci*. 1999; 40:2912–17. [PubMed: 10549652]
- Liu JH, Zhang X, Kripke DF, Weinreb RN. Twenty-four-hour intraocular pressure pattern associated with early glaucomatous changes. *Invest Ophthalmol Vis Sci*. 2003; 44: 1586–90. [PubMed: 12657596]
- Lorenz K, Korb C, Herzog N, et al. Tolerability of 24-hour intraocular pressure monitoring of a pressure-sensitive contact lens. *J Glaucoma*. 2013; 22:311–16. [PubMed: 23524857]
- Mansouri K, Medeiros FA, Tafreshi A, Weinreb RN. Continuous 24-hour monitoring of intraocular pressure patterns with a contact lens sensor: Safety, tolerability, and reproducibility in patients with glaucoma. *Arch Ophthalmol*. 2012; 130:1534–9.
- McLaren JW, Brubaker RF, FitzSimon JS. Continuous measurement of intraocular pressure in rabbits by telemetry. *Invest Ophthalmol Vis Sci*. 1996; 37:966–75. [PubMed: 8631640]
- Melki S, Todani A, Cherfan G. An implantable intraocular pressure transducer: Initial safety outcomes. *JAMA Ophthalmol*. 2014; 132:1221–5. [PubMed: 24970583]
- Mermoud A, Baerveldt G, Minckler DS, et al. Intraocular pressure in Lewis rats. *Invest Ophthalmol Vis Sci*. 1994; 35:2455–60. [PubMed: 8163335]
- Moore CG, Johnson EC, Morrison JC. Circadian rhythm of intraocular pressure in the rat. *Curr Eye Res*. 1996; 15:185–91. [PubMed: 8670727]
- Morrison JC, Jia L, Cepurna W, et al. Reliability and sensitivity of the TonoLab rebound tonometer in awake Brown Norway rats. *Invest Ophthalmol Vis Sci*. 2009; 50:2802–8. [PubMed: 19324849]
- Morrison JC, Johnson EC, Cepurna W, Jia L. Understanding mechanisms of pressure-induced optic nerve damage. *Prog Retin Eye Res*. 2005; 24:217–40. [PubMed: 15610974]
- Morrison JC, Moore CG, Deppmeier LM, et al. A rat model of chronic pressure-induced optic nerve damage. *Exp Eye Res*. 1997; 64:85–96. [PubMed: 9093024]
- Musch DC, Gillespie BW, Niziol LM, et al. Intraocular pressure control and long-term visual field loss in the Collaborative Initial Glaucoma Treatment Study. *Ophthalmology*. 2011; 118:1766–73. [PubMed: 21600658]
- Nickells RW, Howell GR, Soto I, John SW. Under pressure: Cellular and molecular responses during glaucoma, a common neurodegeneration with axonopathy. *Annu Rev Neurosci*. 2012; 35: 153–79. [PubMed: 22524788]
- Nissirios N, Chanis R, Johnson E, et al. Comparison of anterior segment structures in two rat glaucoma models: An ultrasound biomicroscopic study. *Invest Ophthalmol Vis Sci*. 2008; 49:2478–82. [PubMed: 18515586]
- Pang IH, Wang WH, Clark AF. Acute effects of glaucoma medications on rat intraocular pressure. *Exp Eye Res*. 2005; 80: 207–14. [PubMed: 15670799]

- Piccione G, Giannetto C, Fazio F, Giudice E. Influence of different artificial lighting regimes on intraocular pressure circadian profile in the dog (*Canis familiaris*). *Exp Anim*. 2010; 59: 215–23. [PubMed: 20484855]
- Portaluppi F, Smolensky MH, Touitou Y. Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol Int*. 2010; 27:1911–29. [PubMed: 20969531]
- Refinetti R, Lissen GC, Halberg F. Procedures for numerical analysis of circadian rhythms. *Biol Rhythm Res*. 2007; 38:275–325. [PubMed: 23710111]
- Rosenwasser AM. Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiol Behav*. 1993; 53: 1035–41. [PubMed: 8346285]
- Rowland JM, Potter DE, Reiter RJ. Circadian rhythm in intraocular pressure: A rabbit model. *Curr Eye Res*. 1981; 1:169–73. [PubMed: 7297102]
- Samsel PA, Kisiswa L, Erichsen JT, et al. A novel method for the induction of experimental glaucoma using magnetic microspheres. *Invest Ophthalmol Vis Sci*. 2011; 52:1671–5. [PubMed: 20926815]
- Schnell CR, Debon C, Percicot CL. Measurement of intraocular pressure by telemetry in conscious, unrestrained rabbits. *Invest Ophthalmol Vis Sci*. 1996; 37:958–65. [PubMed: 8631639]
- Shareef SR, Garcia-Valenzuela E, Salierno A, et al. Chronic ocular hypertension following episcleral venous occlusion in rats. *Exp Eye Res*. 1995; 61:379–82. [PubMed: 7556500]
- Sommer A, Tielsch JM, Katz J, et al. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. *Arch Ophthalmol*. 1991; 109:1090–5. [PubMed: 1867550]
- Sposato V, Parisi V, Manni L, et al. Glaucoma alters the expression of NGF and NGF receptors in visual cortex and geniculate nucleus of rats: Effect of eye NGF application. *Vision Res*. 2009; 49:54–63. [PubMed: 18938194]
- Todani A, Behlau I, Fava MA, et al. Intraocular pressure measurement by radio wave telemetry. *Invest Ophthalmol Vis Sci*. 2011; 52:9573–80. [PubMed: 22039243]
- Twa MD, Roberts CJ, Karol HJ, et al. Evaluation of a contact lens-embedded sensor for intraocular pressure measurement. *J Glaucoma*. 2010; 19:382–90. [PubMed: 20051894]
- Ueda J, Sawaguchi S, Hanyu T, et al. Experimental glaucoma model in the rat induced by laser trabecular photocoagulation after an intracameral injection of India ink. *Jpn J Ophthalmol*. 1998; 42:337–44. [PubMed: 9822959]
- Valderrama CM, Li R, Liu JH. Direct effect of light on 24-h variation of aqueous humor protein concentration in Sprague-Dawley rats. *Exp Eye Res*. 2008; 87:487–91. [PubMed: 18822284]
- Vidal-Sanz M, Salinas-Navarro M, Nadal-Nicolas FM, et al. Understanding glaucomatous damage: Anatomical and functional data from ocular hypertensive rodent retinas. *Prog Retin Eye Res*. 2012; 31:1–27. [PubMed: 21946033]
- Wang WH, Millar JC, Pang IH, et al. Noninvasive measurement of rodent intraocular pressure with a rebound tonometer. *Invest Ophthalmol Vis Sci*. 2005; 46:4617–21. [PubMed: 16303957]
- Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: A review. *JAMA*. 2014; 311:1901–11. [PubMed: 24825645]
- Yu S, Tanabe T, Yoshimura N. A rat model of glaucoma induced by episcleral vein ligation. *Exp Eye Res*. 2006; 83:758–70. [PubMed: 16707124]

FIGURE 1.

Experimental timeline used to quantify the circadian rhythm of intraocular pressure (IOP) and core body temperature in two groups of animals. Both groups were initially housed in standard light-dark environmental conditions. Animals were trained during *Week 1* to hold still while measuring IOP. An intraperitoneal temperature sensor was implanted (IP surgery) during *Week 2*. Animals recovered from surgery during *Week 3*. Body temperature measurements were then obtained every 5 min until the end of each protocol (gray shaded area). IOP rhythms were measured every two-hours over the course of 26 h at distinct time-points. (A) In group 1, IOP rhythm in light-dark condition was measured at the end of *Week 5*. IOP rhythm was reevaluated in Group 1 after being housed in continuous dim light for 1 week (end of *Week 6*) and 4 weeks (end of *Week 9*). (B) In Group 2, IOP rhythm was evaluated after the animals had been placed in continuous dim light for 7 weeks and was re-evaluated after the animals had been exposed to standard light-dark conditions for 4 weeks.

FIGURE 2.

Daily temperature variations in standard light-dark environmental conditions and continuous dim light for one representative animal from Group 2. The black line is the least-squared cosine fit through the raw temperature data (gray line) and the correlations of determination (r^2) are shown for each graph. The large dark points (blue) connected vertically by the dashed line indicate temperature onset times and the large adjacent points (magenta) indicate temperature peak times. (A) Temperature recorded after 38–45 days exposure to continuous dim light and shows that temperature onset time drifted forward in continuous dim light. (B) Temperature recorded for the first 8 days after the animal was switched to light-dark environmental conditions. Temperature onset time drifted forward for the first four days of re-entrainment and then it consistently occurred at the beginning of the dark phase. (C) Temperature recorded when the animal had re-entrained to the light-dark conditions for four weeks. Temperature onset time in standard light-dark conditions consistently occurred at the beginning of the dark phase and temperature peaked in the middle of the dark phase.

FIGURE 3.

Daily variations of intraocular pressure in two Brown Norway rats (a Group 1 animal and a Group 2 animal) housed in standard light-dark conditions and in continuous dim light. The open circles are the raw (mean $\pm$ standard deviation) intraocular pressure measurements. The cosine fits for intraocular pressure (black solid line) are plotted in either Zeitgeber Time (when animals were kept in light-dark environmental conditions) or Circadian Time (when animals were housed in continuous dim light). In the Group 1 animal, intraocular pressure was measured in (A) light-dark conditions and then measured again after (B) 1 week and (C) 4 weeks of continuous dim light exposure. In the Group 2 animal, intraocular pressure was measured (D) after being housed for 7 weeks in continuous dim light and (E) then after it re-entrained to the standard light schedule.

FIGURE 4.

Relationship between core body temperature (red solid lines) and intraocular pressure (black solid lines) cosine-fit results for all animals in Group 1 (A) in standard light-dark conditions, (B) after 1 week in continuous dim light, and (C) after 4 weeks in continuous dim light. Temperature peak time is indicated by the red dashed line and intraocular pressure peak time is indicated by the black dashed line.

FIGURE 5.

Relationship between core body temperature (red solid lines) and intraocular pressure (black solid lines) cosine-fit results for all animals in Group 2 (A) in standard light-dark conditions, and (B) after 7 weeks in continuous dim light. Temperature peak time is indicated by the red dashed line and intraocular pressure peak time is indicated by the black dashed line.

FIGURE 6.

Relationship between core body temperature (○) and intraocular pressure (×) peak times in standard light-dark environmental conditions (LD), and 1, 4 and 7 weeks of exposure to continuous dim light (LL). Points are color coded by animal. (A) Plotting Zeitgeber Time (clockwise) at a normalized radial distance of 1. In standard light-dark environmental conditions, temperature and intraocular pressure peaked at similar times near the middle of the dark phase. (B–D) Plotting Circadian Time at a radial distance equal to the normalized amplitude (e.g. temperature amplitude in LL divided by the temperature amplitude in LD). The difference between temperature and intraocular pressure peak times was more variable in continuous dim light and amplitude dampened in all animals housed in continuous dim light for 7 weeks.

TABLE 1

Daily variations in core body temperature by lighting condition.

Light condition	Group	Mean $\pm$ SD (95% confidence interval)				Circular statistics		
		Rhythmicity [%]	24-h Mean [mm hg]	IOP range [mm hg]	Resultant vector	Circular variance	Rayleigh's test	
LD	1&2	46 $\pm$ 21* (32–60)	37.2 $\pm$ 0.1 (37.1–37.3)	0.06 $\pm$ 0.11 (0.44–0.74)	0.98	0.02	$p < 0.001$	
1 weeks in LL	1	19 $\pm$ 6* (9–28)	37.0 $\pm$ 0.2 (36.7–37.2)	0.32 $\pm$ 0.03 (0.20–0.42)	–	–	–	
4 weeks in LL	1	10 $\pm$ 2* (7–13)	37.0 $\pm$ 0.2 (36.7–37.4)	0.26 $\pm$ 0.02 (0.20–0.30)	–	–	–	
7 weeks in LL	2	17 $\pm$ 7* (10–23)	37.1 $\pm$ 0.1 (37.0–37.2)	0.34 $\pm$ 0.07 (0.20–0.43)	–	–	–	

Mean core body temperature recorded under all lighting conditions by experimental group. Light condition LD refers to animals being housed in standard light-dark environmental conditions and LL refers to animals that were housed in continuous dim light. Two groups of animals were included in the study; Group 1 ($n=4$ animals) was first housed in LD and transferred to LL for 4 weeks; Group 2 ($n=7$ animals) was housed in LL for 7 weeks and then switched to LD. Rhythmicity refers to the correlation of determination of the cosine fit analysis multiplied by 100%. Circular statistics were used to determine the spread of intraocular pressure peak times around the clock. A mean resultant vector of one and circular variance of zero would suggest that, for all animals, intraocular pressure was at a maximum at the same time. Rayleigh's test yields a 1 if peak times are uniformly distributed around the clock. Circular statistics were not performed for data collected in continuous dim light because core body temperature was anchored to circadian time 18:00 h.

* $p < 0.001$.

TABLE 2

Daily variations of intraocular pressure by lighting condition.

Light condition	Group	Mean $\pm$ SD (95% confidence interval)			Circular statistics		
		Rhythmicity [%]	24-h Mean [mmHg]	IOP range [mmHg]	Resultant vector	Circular variance	Rayleigh's test
LD	1&2	46 $\pm$ 21* (36–55)	22 $\pm$ 3 (20–24)	14 $\pm$ 3 (10–18)	0.96	0.04	$p < 0.001$
1 weeks in LL	1	24 $\pm$ 7* (13–35)	18 $\pm$ 2 (16–21)	8 $\pm$ 1 (6–12)	0.98	0.02	$p = 0.01$
4 weeks in LL	1	30 $\pm$ 14* (8–51)	20 $\pm$ 2 (18–23)	8 $\pm$ 2 (2–14)	0.21	0.79	$p = 0.85$
7 weeks in LL	2	20 $\pm$ 10* (11–30)	22 $\pm$ 2 (20–23)	6 $\pm$ 1 (4–8)	0.11	0.89	$p = 0.92$

Intraocular pressure recorded under all lighting conditions by experimental group. Light condition LD refers to animals being housed in standard light-dark environmental conditions and LL refers to animals that were housed in continuous dim light. Two groups of animals were included in the study; Group 1 ($n=4$ animals) was first housed in LD and transferred to LL for 4 weeks; Group 2 ($n=7$ animals) was housed in LL for 7 weeks and then switched to LD. Rhythmicity refers to the correlation of determination of the cosine fit analysis multiplied by 100%. Circular statistics were used to determine the spread of intraocular pressure peak times around the clock. A mean resultant vector of one and circular variance of zero would suggest that, for all animals, intraocular pressure was at a maximum at the same time. Rayleigh's test yields a 1 if peak times are uniformly distributed around the clock.

* $p < 0.001$