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Influence of Different Artificial Lighting Regimes on Intraocular Pressure Circadian Profile in the Dog (*Canis familiaris*)

Giuseppe PICCIONE[1)], Claudia GIANNETTO[1)], Francesco FAZIO[1)], and Elisabetta GIUDICE[2)]

[1)]*Department of Experimental Sciences and Applied Biotechnology, Laboratory of Veterinary Chronophysiology, Faculty of Veterinary Medicine, University of Messina, Messina 98168, Italy* and [2)]*Department of Veterinary Public Health, University of Messina, Messina 98168, Italy*

Abstract: The present study was undertaken to determine the temporal variation in intraocular pressure (IOP) and if this variation is under circadian clock control. The authors exposed five female and five male Beagles to four different artificial lighting regimes: 12/12 light/dark (L/D) period, 12/12 D/L period, constant light, and constant darkness. IOP was measured at 3 h intervals over a 48-h period. Statistical analysis of the data was performed by multivariate ANOVA, one-way repeated measure ANOVA and by the single cosinor method. Results showed no statistical effect of gender, eye and photoperiod on IOP values. A significant effect of time for each gender and each eye during all lighting regimes was seen, except during constant light, and also robust daily rhythmicity of IOP values in all L/D periods, except during constant light. In conclusion IOP values in the dog show a circadian rhythm and this rhythm is driven by a central pacemaker.

Key words: circadian rhythm, dog, eye, intraocular pressure, lighting schedules

Introduction

The mammalian circadian clock regulates the temporal organization of biological and physiological functions [13, 48]. The mammalian eye shows circadian rhythms in various processes at all levels of organization from the molecular, such as the release of melatonin and dopamine, through the cellular, such as retinomotor movements and rod outer segment phagocytosis, whole organ, such as tear production and intraocular pressure (IOP), and visual system levels, such as visual pigment and visual sensitivity [2, 7, 9, 18, 29, 36, 47].

Since the introduction of applanation tonometers, IOP has been measured more easily in veterinary medicine [8]. Measurement of IOP remains a routine investigation in eye examinations and is accepted as a fundamental parameter of ocular health and disease; it is important in the diagnosis and management of glaucomatous conditions, uveitis and in the postoperative management of corneal, lenticular and vitreoretinal diseases [12, 22]. IOP is maintained via a continuous secretion of aqueous humor from the ciliary epithelium of the ciliary body and drainage out of the eye through the Canal of Schlemm and the uveoscleral pathway. Changes in the rate of

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Address corresponding: C. Giannetto, Department of Experimental Sciences and Applied Biotechnology, Laboratory of Veterinary Chronophysiology, Faculty of Veterinary Medicine, University of Messina, polo universitario dell'Annunziata, Messina 98168, Italy

secretion of aqueous humor and/or resistance to its drainage result in changes in IOP [5]. Temporal variations of IOP are driven by the suprachiasmatic nucleus (SCN), which controls the activity of the sympathetic and parasympathetic ocular innervations [3]. These innervations are responsible for controlling the production (β-adrenergic system) and outflow (α_1-adrenergic, parasympathetic system, prostaglandin) of aqueous humor [20].

IOP circadian variations have been studied in numerous species, the phase and amplitude of these rhythms differ among them. In humans, the data reported are inconsistent. Some authors have reported higher IOP values at night than during the day, with a nocturnal peak value [41], while Jaén-Diaz *et al*. [23] found higher IOP values in the morning than in the evening. In the mouse, rabbit, rat, chicken, and marmoset, the 24-h IOP pattern is biphasic when the animals are maintained on a 12/12 L/D cycle [1, 31, 33, 34, 42, 45]. In the rabbit, it was found that there was a tendency toward an increase in the IOP in the morning with a sharp decline in the afternoon [24]; in the marmoset IOP was higher during the dark phase than the light phase [34], but IOP was not sampled at sufficient intervals to determine the precise phase or amplitude. In the horse, an IOP peak was observed at the end of the daytime and a trough during the nighttime [4]. In the cat, the highest values of IOP were observed during the night [15].

In the dog, a diurnal effect has been detected in both normal and glaucomatous Beagles, and IOP was slightly higher in the morning than in the early evening [19].

In nocturnal species such as rats, cats, and rabbits, IOP levels increase during the night, whereas in diurnal species such as dogs, monkeys, and humans, IOP peaks were reported during the day [15, 19, 27]. In agreement with these previous studies, we observed an increase of IOP levels in a diurnal species, the horse, during the daytime [4].

Previous investigations have shown that the IOP rhythm is entrained by an light/dark (L/D) cycle and persists in constant darkness, demonstrating a circadian component controlled by an endogenous pacemaker [11].

Understanding the circadian change in IOP, as well as its scope and potential factors indicating change, is very significant not only from a research viewpoint but also from a clinical perspective in terms of diagnosis and management of ocular diseases.

In this study, we examined temporal variation of IOP in healthy female and male Beagles dogs maintained under L/D cycles or constant lighting conditions (constant darkness and constant light), to demonstrate the existence of 24-h variation in the IOP level in dogs and to test whether the identified temporal variation is under the control of the circadian clock and how it is affected by the environmental lighting regime.

Materials and Methods

Animals and housing

Ten clinically healthy Beagle dogs (*Canis familiaris*), five females (mean age: 5 ± 1 year old; mean body weight: 13.5 ± 1 kg) and five males (mean age: 5 ± 1 year old; mean body weight: 15.0 ± 1 kg), were used. They were housed individually at an indoor temperature and humidity of 18–21°C and 50–60 Rh%. Ambient temperature and relative humidity for each experimental day were continuously recorded with a data logger (Gemini, Chichester, UK). The shell of the boxes allowed the visual isolation of each dog from cospecifics and avoided the social entrainment of circadian behavioural rhythms [14]. All dogs received standard feeding (22.5 g/kg for each dog of a certified dog diet) provided at 10:00 each day. Water was available *ad libitum*. General animal care was carried out by professional staff not associated with the research team at different times of the day to avoid entraining signals. All the work described here complied with current regulations covering animal experimentation in Italy.

Experimental design

Prior to the study, complete clinical and ophthalmic examinations were performed on all dogs to determine their health status. Ocular examination included direct ophthalmoscopy, Schirmer tear test (STT) I, applanation tonometry, biomicroscopy, flourescein staining, and electroretinography (ERG). All animals were free of signs of corneal or conjunctival disease, had no history of ocular diseases and no showed individual differences in IOP values.

All dogs were exposed to four different artificial lighting regimes in the individual boxes, without windows, to avoid natural lighting, and for 3 days prior to each L/D schedule the animals underwent the same pattern of daily activity [39]: in the first schedule (12/12 L/D period) light timers were set to maintain a L/D cycle with 12 h of light and 12 h of darkness each day (600 lx; lights on at 07:00); during the second schedule (12/12 D/L period), the L/D cycle was delayed by 12 h (lights on at 19:00 and off at 07:00–12/12 D/L period); during the third schedule (24/0 L/D period) the lights were turned on for all of the experimental period; during the fourth schedule (0/24 L/D period) all animals were housed in constant darkness; the last period was used to re-establish the 12/12 L/D period.

For each dog, the IOP was measured at 3-h intervals over a 48-h period (starting at 08:00 on day 1 and finishing at 08:00 on day 3).

Lighting was uniformly diffused throughout the animal box and provided sufficient illumination for good house-keeping practices, adequate inspection of animals, safe working conditions for personnel, and for the well-being of the animals. Light was provided by cool daylight fluorescent tubes (FH HE/860 Lumilux T5, Osram GmbH, Milano, Italy) placed in the middle of the box at 3 m height from the floor. The light intensity was measured by a photometer (PCE-172, PCE Group S.R.L, Lucca, Italy). Dim red light (<3 lx, 15 W Safelight lamp filter 1A, Kodak Spa, Milano, Italy) was used for data collection, feeding, and general animal care during the dark phase of the L/D, D/L periods and in D/D period. At the end of the study, a complete ophthalmic examination (slit-lamp biomicroscopy and direct and indirect ophthalmoscopy) was performed on all dogs to determine the health status of their eyes.

IOP assessment

IOP was recorded for both eyes in each animal. The first eye tested was randomly chosen and recorded. Local anaesthetic (0.4% oxibuprocaine chlorhydrate; Novesina, Novartis Farma, Rome, Italy) was instilled a few seconds before the test, and IOP was measured using a Tono-Pen applanation tonometer (Tono-Pen XL, Reichert, Inc., Depew, NY, USA) by the same person. The Tono-Pen applanation tonometer is generally accepted as the most satisfactory tonometer for canine clinical use [26]. The Tono-Pen tonometer was used and maintained in accordance with the manufacturers recommendations. It was gently placed in contact with the cornea, and a sanitized Ocu-Film tip cover (Tono-Pen XL, Reichert, Inc.) was used to minimize the risk of cross-contamination. Three measurements were made at each time point, and mean values were calculated. Measurements were repeated until the instrument error was <5%. In dogs, the influence of body position on IOP levels has been well documented by Broadwater *et al*. [6]. To avoid positional changes in IOP, we always performed the measurement in the sitting position.

Statistical analysis

All the results are expressed as mean ± SD. Data were normally distributed ($P<0.05$, Kolmogorov-Smirnov test). Multivariate analysis of variance (MANOVA) was used to compare IOP values obtained in female and male, left and right eyes and to determine the influence of photoperiod on IOP values during protocol testing. One-way repeated measure ANOVA was used to determine the statistically significant effect of time on IOP in each eye in the different L/D schedules. P values <0.05 were considered statistically significant. The data was analyzed using the software STATISTICA 7 (StatSoft Inc., Tulsa, OK, USA).

In addition, we applied a trigonometric statistical model to the average values of each time series, so as to describe the periodic phenomenon analytically, by characterizing the main rhythmic parameters according to the single cosinor procedure [32]. Four rhythmic parameters were determined: mean level, amplitude, acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). For each parameter, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (9 data points), the amplitude of a rhythm was calculated as half the range of oscillation, which in turn was computed as the difference between peak and trough. Rhythmic robustness was computed as a percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity [38]. Robustness greater than 35% is above the noise level and indicates statistically significant rhythmicity.

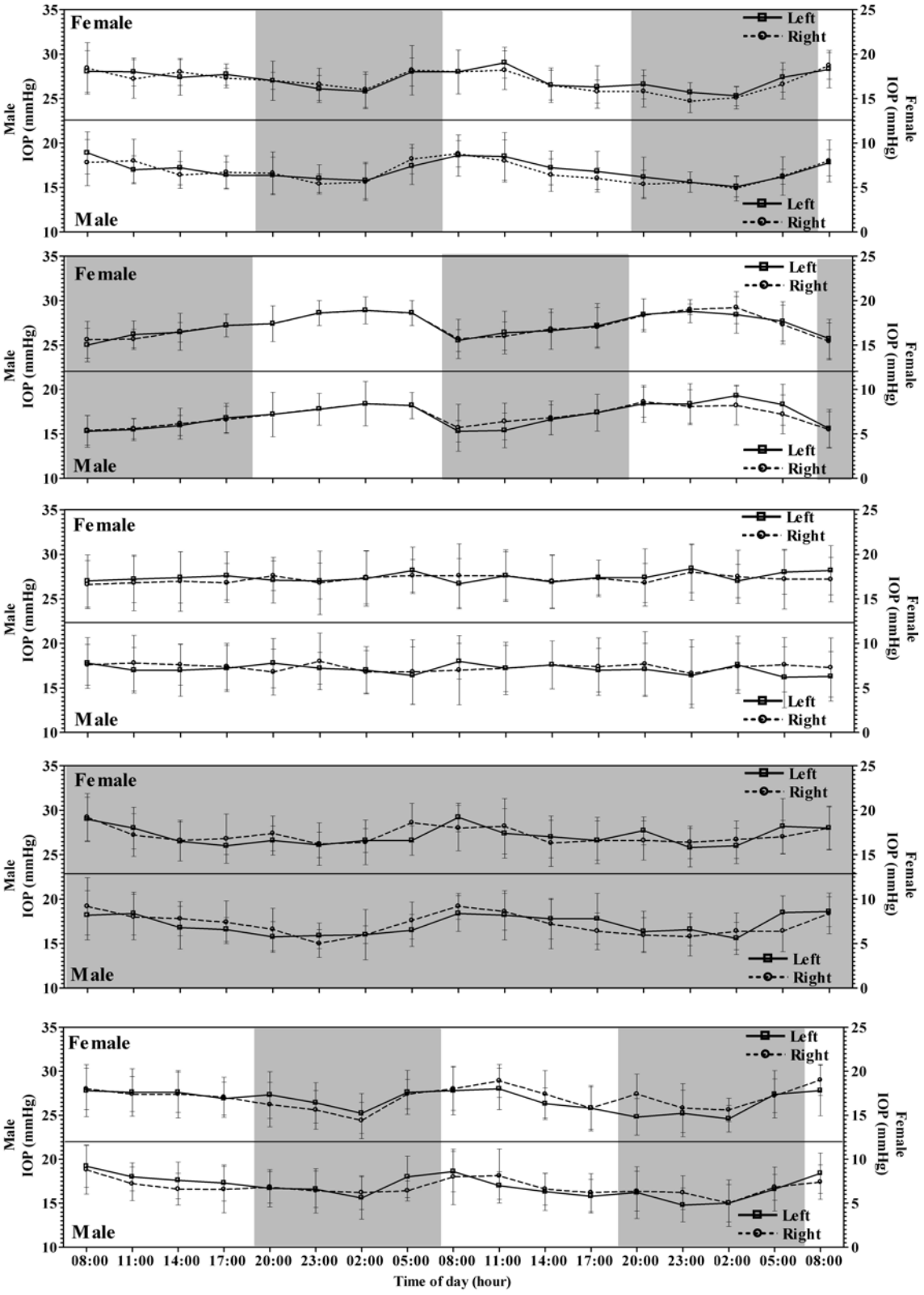

Fig. 1. Daily rhythms of IOP in female (n=5) and male (n=5) dogs during the L/D period, D/L period, L/L period, D/D period, and L/D period. Each point represents the mean (± SD) of IOP of the left and right eyes. Grey bars indicate the dark phase of the 48 h photoperiod.

Table 1. Statistical analyses (ANOVA and Cosinor) of both eyes in five female and five male dogs in the five L/D schedules

	Period	Eye		$F_{(8,32)}$	*P*	Robustness	Φ	A
Female	L/D	Right	Day 1	2.32	0.04	74.80	6.00	1.87
			Day 2	3.81	0.003	84.80	4.30	1.63
		Left	Day 1	2.31	0.04	89.50	4.30	1.60
			Day 2	2.48	0.03	59.50	5.30	1.40
	D/L	Right	Day 1	2.89	0.01	95.80	20.24	1.57
			Day 2	2.70	0.02	79.70	18.33	1.67
		Left	Day 1	4.14	0.001	90.00	20.01	1.62
			Day 2	2.30	0.04	90.09	18.23	1.56
	L/L	Right	Day 1	0.12	0.99	No rhythmicity		
			Day 2	0.11	0.99			
		Left	Day 1	0.22	0.98	No rhythmicity		
			Day 2	0.71	0.67			
	D/D	Right	Day 1	2.40	0.03	35.00	3.43	1.61
			Day 2	2.29	0.04	55.60	4.48	1.05
		Left	Day 1	2.63	0.02	76.40	6.00	2.04
			Day 2	2.28	0.04	54.40	5.00	1.86
	L/D	Right	Day 1	2.64	0.02	37.00	4.48	0.94
			Day 2	2.53	0.02	59.00	3.42	1.72
		Left	Day 1	2.24	0.04	80.50	3.48	1.50
			Day 2	2.43	0.03	52.50	4.42	1.62
Male	L/D	Right	Day 1	2.49	0.03	95.50	4.29	1.43
			Day 2	2.82	0.01	92.40	5.41	1.95
		Left	Day 1	2.37	0.04	66.40	4.54	1.49
			Day 2	2.47	0.03	93.30	7.02	1.62
	D/L	Right	Day 1	2.47	0.03	98.10	20.46	1.38
			Day 2	2.28	0.04	89.30	17.33	1.46
		Left	Day 1	4.09	0.001	98.20	20.34	1.44
			Day 2	6.65	0.0001	94.60	19.15	1.86
	L/L	Right	Day 1	0.14	0.99	No rhythmicity		
			Day 2	0.07	0.99			
		Left	Day 1	0.23	0.98	No rhythmicity		
			Day 2	0.44	0.88			
	D/D	Right	Day 1	2.47	0.03	90.50	5.45	1.93
			Day 2	2.37	0.03	94.20	4.45	1.35
		Left	Day 1	2.90	0.01	88.40	5.54	1.46
			Day 2	2.41	0.03	90.50	4.14	1.22
	L/D	Right	Day 1	2.40	0.03	50.60	5.46	1.04
			Day 2	2.27	0.04	59.00	6.02	1.26
		Left	Day 1	2.23	0.05	60.00	5.28	1.47
			Day 2	2.46	0.03	69.80	5.21	1.71

Results

Left and right eyes both in female and male dogs showed the same trend (Fig. 1).

Multivariate analysis showed no statistically significant differences in IOP values for female and male dogs [$F_{(1,1680)}$=1.1; *P*=0.29], left and right eyes [$F_{(1,1680)}$=0.1; *P*=0.79], and the different photoperiods [$F_{(4,1680)}$=2.3; *P*=0.06]. One-way ANOVA showed a significant effect of time on IOP for each eye and each gender in all L/D schedules ($P<0.05$), except for both eyes and gender during the L/L period (Table 1).

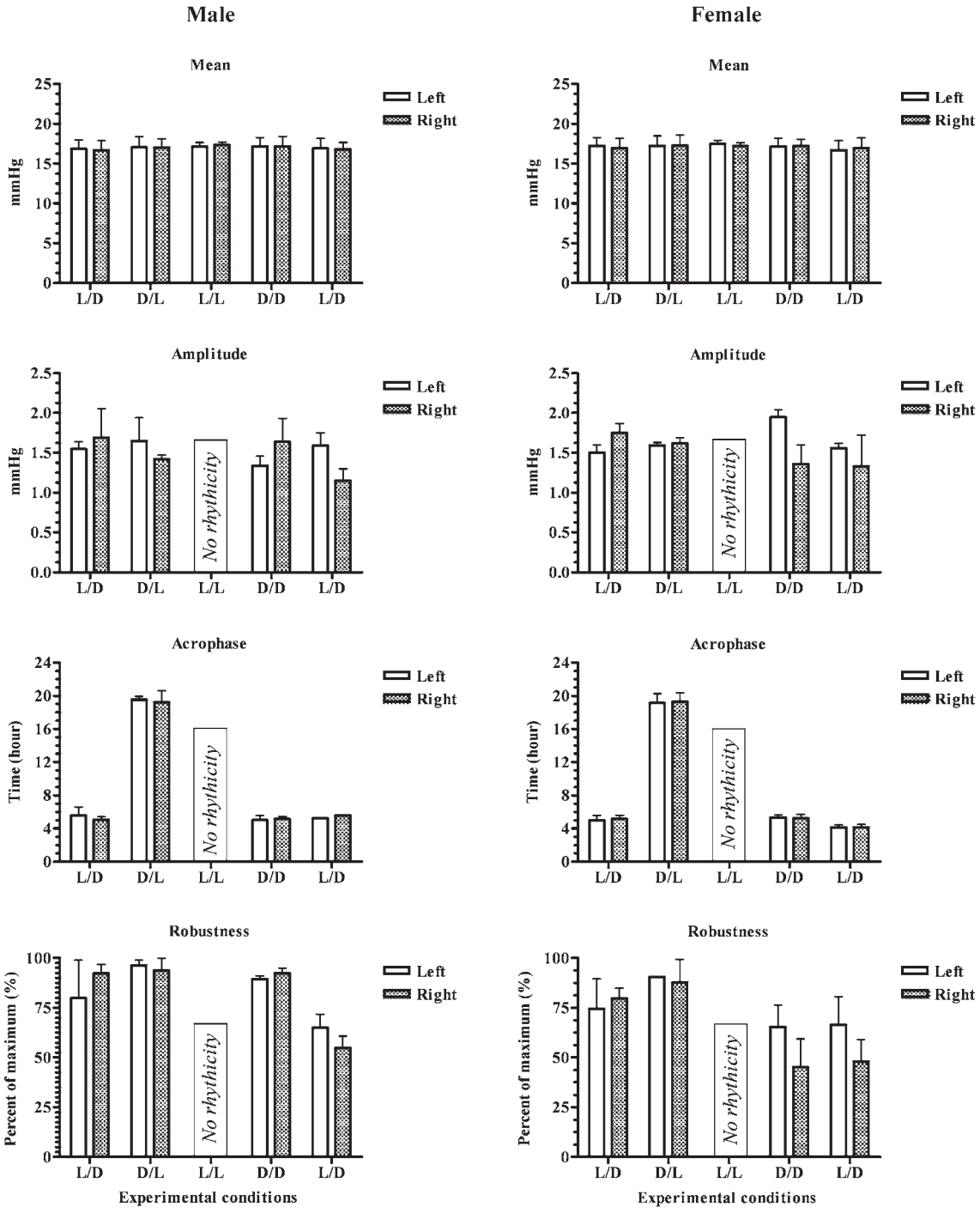

Fig. 2. Analysis of four rhythmic parameters in 48-h records of IOP in left or right eyes. Each bar corresponds to the mean (± SD) of five dogs.

Application of the periodic model and the statistical analysis of the cosinor procedure throughout the time series studied under the different experimental conditions, allowed us to ascertain the periodic pattern of IOP in both eyes (Fig. 2).

Robust daily rhythmicity was exhibited by IOP during

all experimental photoperiods except during the L/L period, in both eyes. All the rhythmic parameters showed the acrophase during the scotophase, except in the first day of the D/L cycle, in which the acrophase was observed between 20:24 and 20:46 (Table 1).

Discussion

The results of the present study were consistent with the reference range previously reported for the dog [20]. Left and right eyes exhibited the same trend of IOP values in both genders, as previously observed in the cat, in which male diurnal IOP does not differ from that of either spayed or intact female cats [15], while a different trend was observed between the different lighting schedules. The results also showed a statistically significant effect of time on IOP values for each eye and each gender under all experimental conditions, except during the constant light period, as previously observed for STT I values in dogs [37].

A robust circadian rhythm was observed in IOP values in the 12/12 L/D period in both eyes and in both genders with a nocturnal acrophase nearly at the end of the scotophase (between 04:30 and 06:50). When the L/D cycle was delayed by 12-h, the circadian rhythm of IOP was delayed too, even if some days were necessary to bring the acrophase from diurnal to nocturnal. In both genders, during the first day of monitoring the acrophase was observed during the photophase (between 20:24 and 20:46), while during the second day it was observed during the scotophase (between 17:33 and 19:15). In humans and rabbits [27] it is hypothesized that the circadian elevation of IOP is due to an increase in general physiological activities. However, the circadian increase of dog IOP in the dark was independent of physiological activity. Dow *et al*. [17] suggested that the dog total activity count can vary considerably depending on the day of the week and is influenced by owners' activity. The existence of a nocturnal acrophase also excludes the influence of the daily rhythm of heart rate and blood pressure on the daily rhythm of IOP. In a previous study, dogs subjected to an artificial 12/12 L/D cycle showed diurnal acrophases in heart rate and blood pressure [35].

Rhythmicity was lost under constant light, but appeared again in constant darkness with acrophases similar to those observed in the 12/12 L/D period. When the 12/12 L/D cycle was re-established the circadian rhythm of IOP values showed acrophases similar to those observed in the first 12/12 L/D period, but with a lower robustness value.

The persistence of an IOP circadian rhythm in constant dark and the loss of this rhythm under constant exposure to light was previously observed in rabbits, rats and mice [28, 30, 31, 43], and was attributed to the existence of an endogenous pacemaker entrained by the lighting regime. Several investigation evinced deep alterations and/or suppression of circadian rhythms upon exposure to constant light [4, 16]. However, other factors may be involved in the abolition of IOP rhythms in constant light. For instance, dogs might modify their body posture to avoid disturbance from light exposure. Furthermore, the effects of constant light on other parameters of aqueous humor dynamics, such as outflow resistance and episcleral venous pressure are unknown. The absence of statistically significant differences due to different photoperiods permits us to exclude the influence of the natural papillary light reflex, such as mydriasis and myosis, on the circadian rhythm of IOP. The application of mydriatics resulted in a significant elevation of IOP in cats and humans, probably due to a decrease in aqueous outflow [21, 44]. Also, the presence of the acrophase of IOP circadian rhythm during the photophase in the D/L period, effectively excludes the influence of the natural papillary light reflex on IOP circadian rhythm regulation.

Many hypothesise have been proposed to explain the exact mechanism regulating the IOP diurnal variation. For example, the change of endogenous hormones may play a role in the circadian elevation of IOP. It has also been hypothesized that the diurnal variation in IOP is largely a reflection of the diurnal variation in plasma cortisol. It has been suggested that the aqueous humor concentration increases in tandem with to the circadian elevation of IOP [27]. Human studies also suggest that endogenous catecholamines increase aqueous flow in the daytime, when the IOP is generally high [25, 40, 46]. Therefore, it has been hypothesized that aqueous production may be dependent on beta adrenergic tone that is mediated by circulating adrenaline levels. Melatonin

levels may also be associated with increase of IOP [49]. Plasma melatonin originates mainly from pineal melatonin which is synthesized in a circadian manner. The origin of melatonin in the aqueous humor is unclear. The small molecule of melatonin is capable of diffusing from the plasma into the aqueous humor. Aqueous humor melatonin may also be produced locally in the anterior segment [10]. This may explain why, under constant light the IOP values did not had a wide oscillation during the two days of monitoring in all subjects, and showed the lowest standard deviation of the mean daily value compared with the others experimental periods. It may also explain why the acrophase was always observed during the scotophase. Even thought the effect of melatonin on dog IOP need to be tested in order to prove that it modulates canine IOP, studies on the rabbit have reported that melatonin does not play a major, direct role in the circadian elevation of IOP [27].

In conclusion the results demonstrate that IOP values in healthy dogs show a circadian rhythm and that this rhythm might be driven by a central pacemaker entrained by the L/D cycle. Also when the canine eye is exposed to constant light the circadian IOP pattern is disrupted. Therefore, IOP is one of the variouis physiological and behavioural systems that are controlled by an internal oscillating mechanism. Excluding many factors that may influence IOP, such as activity, heart rate and blood pressure, we hypothesize that IOP daily rhythm is directly controlled by the nervous system.

Thus, it is important to take to in consideration that changes in IOP observed in the same patient at different clinical examinations may be due to the circadian rhythm rather than to a true change in the mean IOP. Monitoring of the diurnal IOP may be necessary in some cases, if the clinician relies, even partially, on the level of IOP when making a decision on patient management.

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