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Alterations of Locomotor Activity Rhythm and Sleep Parameters in Patients With Advanced Glaucoma

María Florencia Lanzani,[1,2] Nuria de Zavalía,[1] Héctor Fontana,[2] María Ines Keller Sarmiento,[1] Diego Golombek,[3] and Ruth E. Rosenstein[1]

[1]Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, Department of Human Biochemistry, School of Medicine, University of Buenos Aires/CEFyBO, CONICET, Buenos Aires, Argentina, [2]Glaucoma Division, "Santa Lucía" Ophthalmological Hospital, Buenos Aires, Argentina, [3]Laboratory of Chronobiology, National University of Quilmes/CONICET, Pcia. de Buenos Aires, Argentina

The aim of this study was to evaluate the effect of advanced glaucoma on locomotor activity rhythms and related sleep parameters. Nine normal subjects and nine age-matched patients with bilateral advanced primary open-angle glaucoma, >10 yrs since diagnosis, were included in this observational, prospective, case-control study. Patients were required to record the timing and duration of their sleep and daily activities, and wore an actigraph on the wrist of the nondominant arm for 20 d. Activity rhythm period, MESOR (24-h time-series mean), amplitude (one-half peak-to-trough variation), and acrophase (peak time), plus long sleep episodes during the wake state, sleep duration, efficiency, and latency, as well as mean activity score, wake minutes, and mean wake episodes during the sleep interval were assessed in controls and glaucomatous patients. Glaucomatous patients exhibited significant decrease in nighttime sleep efficiency, and significant increase in the mean activity score, wake minutes, and mean wake episode during the night. These results suggest that alterations of circadian physiology could be a risk to the quality of life of patients with glaucoma. (Author correspondence: ruthr@fmed.uba.ar)

Keywords: Actigraphy, Circadian rhythms, Human glaucoma, Melanopsin, Retinal Ganglion cells, Sleep quality

INTRODUCTION

Primary open-angle glaucoma (POAG) represents a chronic, slowly progressive optic neuropathy, characterized by degeneration of retinal ganglion cells (RGCs), progressive excavation of the optic nerve head, and a distinctive pattern of visual field defects. The disease is multifactorial in origin and is associated more closely with elevated intraocular pressure (IOP), resulting in the main from reduced drainage of aqueous humor.

Circadian rhythms are controlled by a biological clock that in humans is located in the suprachiasmatic nuclei (SCN) and has a period of ~24 h (Moore & Silver, 1998). Because SCN neurons contain an internal pacemaker that generates an endogenous rhythmic electrical activity (Buijs & Kalsbeek, 2001), changes supervised by the SCN occur even in the absence of external stimuli. Nevertheless, the SCN are influenced by environmental changes, especially by the light-dark cycle (Golombek & Rosenstein, 2010). The inherited period of the human circadian clock is not precisely 24 h (Czeisler & Gooley, 2007); however, the light-dark and other cyclic environmental and behavioral clues modify the period to precisely 24 h, thereby supporting the circadian daytime activity–nocturnal sleep routine, among many other biological rhythms (Duffy & Czeisler, 2009; Klerman et al., 1998).

Although the eye has been largely recognized as the organ of sight (in man and animals), over the past decade, a second role for the eye has been uncovered. Even in the absence of "cognitive" vision, the eye can serve as a sensor for ambient lighting, akin to the light meter in a camera (Van Gelder, 2003). A host of light-regulated functions, including entrainment of circadian clocks, suppression of activity by light, photic suppression of pineal melatonin synthesis, and pupillary light responses, are retained in animals that are blind as a result of mutations causing complete or near-complete degeneration of the classical photoreceptors, the rods and cones (Freedman et al., 1999; Keeler, 1927; Lucas et al., 1999, 2001). These light-regulated functions are

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María Florencia Lanzani and Nuria de Zavalía contributed equally to this work.

Address correspondence to Ruth E. Rosenstein, Departamento de Bioquímica Humana, Facultad de Medicina, UBA, Paraguay 2155, 5°P, (1121), Buenos Aires, Argentina. Tel.: +54-11-4508-3672 (ext. 37); Fax: 54-11-4508-3672 (ext. 31); Email: ruthr@fmed.uba.ar

controlled by nonclassical retinal photoreceptors, because animals lacking RGCs lose circadian photoresponses, behavioral masking, and pupillary light responses (Wee et al., 2002). The discovery of intrinsically photosensitive RGCs has given nonvisual phototransduction an anatomical basis (Berson et al., 2002; Hattar et al., 2002; Provencio et al., 2002). A subtype of RGCs (mRGCs) containing a novel photopigment, melanopsin, responds to light independently of the input from rods and cones, and project their axons through the retinohypothalamic tract to the SCN. A strong body of evidence supports that mRGCs are involved in modulating circadian rhythms (Mrosovsky & Hattar, 2003; Panda et. al, 2002; Ruby et al., 2002). In addition, mRGCs send monosynaptic projections to the olivary pretectal nucleus, responsible for the pupil light reflex (PLR) (Hattar et al., 2006). In that sense, although it was originally assumed the light-evoked neural signals driving the PLR originated exclusively from rods and cones, it was later shown the mRGCs significantly contribute to pupil constriction (Kardon et al., 2009; Lucas et al., 2003; Nissen et al., 2011). Most studies on glaucoma have focused on RGCs involved in conscious visual functions, and have found that chronic increase in IOP causes progressive loss of these cells. However, clinical studies in glaucomatous patients show a high prevalence of sleep-disordered breathing, characterized by snoring, excessive daytime sleepiness, and insomnia accompanied by large swings in blood pressure and repetitive hypoxic periods during sleep (Onen et al., 2000). Moreover, an abnormal light-induced nocturnal melatonin suppression in glaucoma patients was recently described (Pérez Pico et al., 2010), and afferent pupillary defects during the early stages of the disease were reported (Kalaboukhova et al., 2007; Kohn et al., 1979). In addition, it was recently shown the post-illumination pupil response is dysfunctional in patients with early, moderate, or severe glaucoma (Feigl et al., 2011).

Involvement of mRGCs in glaucoma has been postulated, and two studies in animal models of glaucoma demonstrate loss of mRGCs (Drouyer et al., 2008; Wang et al., 2008). These clues imply that mRGCs, as other RGCs, can be damaged in glaucomatous patients. However, other studies have shown that mRGCs may be spared in some animal models of chronic ocular hypertension (Jakobs et al., 2005; Li et al., 2006). We have recently demonstrated in an experimental rat model of glaucoma induced by chronic injections of chondroitin sulfate (CS) into the eye's anterior chamber (Belforte et al., 2010) that a similar decrease in the number of RGCs projecting to the superior colliculus (which are involved in the image-forming visual system) and of mRGCs (which participate in the non–image-forming visual system) occurs in glaucomatous eyes (de Zavalía et al., 2011). In addition, we have demonstrated that experimental glaucoma induces significant decrease in the afferent pupil light reflex, and alterations in light-induced nocturnal melatonin suppression (de Zavalía et al., 2011). These results indicate that experimental glaucoma can affect the non–image-forming visual system. Notwithstanding, further studies are warranted to clarify the involvement of mRGCs in the natural history of human glaucoma, and to investigate the occurrence of circadian rhythm abnormalities in patients with advanced glaucoma. In that context, and taking into account that there is currently no formal description of behavioral circadian rhythms in glaucomatous patients, the aim of the present report was to analyze if the circadian rhythm in locomotor activity and the sleep-wake cycle, estimated by wrist actigraphy, is altered in advanced stages of glaucoma.

METHODS

Nine patients recruited from the "Santa Lucía" Ophthalmological Hospital with bilateral advanced POAG ≥10 yrs since diagnosis (denoted numbers 1 to 9), and nine normal control subjects (denoted numbers 10 to 18) were included in this prospective pilot observational, case-control study. For this study, advanced glaucoma was considered as a combination of both an optic nerve with a vertical cup to disc ratio >.8 and an altered visual field, defined as a mean deviation worse than −12 db. Nine healthy participants without ocular disease served as the age-matched normal control group and were randomly selected from spouses, friends, and relatives of eye clinic patients or persons from the university staff. Control participants had no morphologic or functional damage indicating glaucoma. Subjects with neurologic and motor disorders, obesity, sleep apnea, or those who used psychoactive agents were excluded, as well as those with nocturnal or shiftwork schedules. All subjects provided written informed consent. Formal approval by the "Santa Lucía" Ophthalmological Hospital Ethics Committee was obtained. The design of the study protocol adhered to the tenets of the Declaration of Helsinki for biomedical research and conformed to international and ethical standards of this journal (Portaluppi et al., 2010).

Ophthalmologic Examinations

Each subject underwent complete ophthalmologic examination. The clinical history and logarithm of the minimum angle of resolution (logMAR) best-corrected visual acuity (BCVA) were recorded, and slit-lamp biomicroscopy of the anterior segment, gonioscopy, Goldmann applanation tonometry, central corneal ultrasonic pachymetry, and ophthalmoscopy of the posterior segment were performed. Patients were excluded if they had a BCVA >+ 1.00, a refractive error >5 diopters equivalent sphere or 3 diopters of astigmatism, or a media opacity such as cataract that prevented ocular examination. The visual field analysis was performed with a Humphrey Field Analyzer II (model 750; Carl Zeiss Meditec, xx, xx) Q2 with the SITA (Swedish Interactive Threshold Algorithm) standard 24-2 program. We included only reliable visual

fields (fixation losses, false positives, and false negatives <25%) in phakic and pseudophakic patients. At least two reliable standard automated perimetry (SAP) tests were performed. Near addition was added to the refractive correction. Control subjects underwent the exact same clinical evaluation as the glaucoma patients.

Locomotor Activity and Sleep/Wake Recordings

Patients were required to record daily activities and the timing and duration of their sleep. Each day, the subject would additionally log waking time, bedtime, number of nighttime awakenings, naps, physical activity prior to sleep, and number of caffeinated beverages or alcoholic drinks consumed. They were also required to keep their daily routine. Moreover, upon enrollment, each subject was provided with an actigraph, a small, light-weight, watch-sized device (Micromini Motionlogger Actigraph; Ambulatory Monitoring, Ardsley, NY, USA) that was worn on the wrist of the nondominant arm continuously for 20 d. This instrument measures the number of movements above threshold intensity, records the amount of movement every 1 min, and stores the information. At the end of the study, the subject's activity was evaluated using automated analysis Action-W 2 software (Ambulatory Monitoring). Locomotor activity was measured with the Zero Crossing (ZCM) mode operation. To differentiate sleep/wake behavior, the Cole-Kripke algorithm (Cole & Kripke, 1988) was used. This method evaluates the number of wrist motions per minute plus a weighted value of motions in the previous and subsequent 2 min and compares this with a threshold value. When 5 consecutive min are scored as sleep, that time is scored as the beginning of a sleep period. Before being monitored, each subject answered a sleep quality questionnaire and a sleep diary from the National Sleep Foundation (http://sleep.buffalo.edu/sleepdiary.pdf).

Statistical Analysis

Cosinor analysis (Bingham et al., 1982) was performed on the time series made up of movement data derived from actigraphy to assess the characteristics of the circadian activity rhythm. Cosinor analysis with an assumed a prior period of 24 h was performed using a specific software for time series (El Temps, Barcelona, Spain) to provide three standard parameters: (i) MESOR (the statistical estimate of average activity over the 24-h period), (ii) amplitude (one-half of the peak to nadir difference), and (iii) acrophase (time of the daily peak activity referenced to local midnight). In all cases, an amplitude significantly different than zero was determined for the 24-h component of the locomotor activity data ($p < .05$), evidencing significant fit of the data to the Cosinor model. Data analysis was assessed by operators masked to the ocular health conditions of each subject.

Q3 Action W-2 software divides the 24-h segment of an actigraphic record into "Up" (active) and "Down" (inactive) intervals by fitting a square wave to the data. Down intervals represent the major sleep period of the day, when subjects are in bed and asleep or trying to sleep. Up intervals represent the times between successive Down intervals. "Long wake episode" and "long sleep episode" are defined as wake or sleep episodes ≥10 min calculated with Action W-2 software. Sleep efficiency was calculated as [100 × (sleep min/Down interval duration)], whereas sleep latency was calculated as the duration in minutes to start the first 20-min block scored as sleep during the Down interval. Wake episodes (WEs) are defined as the number of blocks of contiguous wake epochs, whereas sleep episodes (SEs) are defined as the number of blocks of contiguous sleep epochs. Differences between control subjects and glaucomatous patients were evaluated by Student's *t* test.

RESULTS

Demographic and clinical characteristics of control and glaucomatous patients included in this study are listed in Table 1. Of the nine control subjects, 44% were women, whereas in the group of glaucomatous patients, 55% were women. Mean ± SEM age of the glaucomatous patient was 67 ± 1.9 (range: 60–76) yrs, whereas in the control group, mean age ± SEM was 65.7 ± 1.0 (range: 60–71) yrs. The average age was not significantly different between the control and glaucoma groups ($p = .46$). The follow-up time from POAG diagnosis was 12.7 ± .6 (range: 10.7–14.9) yrs. Ophthalmologic data from both groups are summarized in Table 2. Most eyes in the glaucoma group had trabeculectomy and/or were under topical treatment with prostaglandin drops and a combination

TABLE 1. Demographic data from control subjects and glaucomatous patients

Patient no.	Age (yrs)	Sex (F/M)	Follow up from diagnosis (yrs)	Medical history
1	63	F	11.3	HYT
2	76	F	12.0	—
3	74	F	10.7	OST
4	64	M	15.4	HTA
5	63	F	12.5	HTA
6	60	M	14.9	—
7	73	M	13.3	HTA
8	64	F	12.4	—
9	70	M	10.8	—
10	62	M	—	—
11	71	M	—	—
12	65	M	—	—
13	63	F	—	—
14	65	M	—	—
15	64	M	—	—
16	65	F	—	—
17	70	F	—	OST
18	67	F	—	—

Control subjects: patients 10 to 18; glaucomatous patients: patients 1 to 9. Patient 1 was medicated with levothyroxine to be euthyroid at the time of the study.
M = male; F = female; HTA = arterial hypertension; HYT = hypothyroidism; OST = osteoporosis.

TABLE 2. Ophthalmologic outcomes of control subjects and glaucomatous patients

Patient no.	Ocular history	Optic Disc RE	Optic Disc LE	BCVA (LogMar) RE	BCVA (LogMar) LE	IOP (mm Hg) RE	IOP (mm Hg) LE	HVF MD (dB) RE	HVF MD (dB) LE	HVF PSD (dB) RE	HVF PSD (dB) LE	Topical glaucoma medication
1	—	.8	.8	.00	.00	21	20	25.76	23.86	5.64	12.93	Timolol, dorzolamide, prostaglandins
2	ALT RE	.9	.9	.00	.00	21	16	26.76	16.86	8.84	11.48	Timolol, dorzolamide, prostaglandins
3	—	.9	.8	1.00	.10	13	13	28.76	11.86	5.64	9.93	Timolol, dorzolamide, prostaglandins
4	—	1.0	.8	.60	.18	16	17	25.33	11.17	8.86	11.50	Timolol, dorzolamide, prostaglandins
5	TBC BE	.9	1.0	1.00	.95	11	11	29.67	33.79	5.20	2.69	—
6	TBC BE	.9	.9	.00	.00	16	16	29.73	23.55	10.41	12.73	Timolol, dorzolamide, prostaglandins
7	SLT RE	.9	.8	.40	.00	17	17	16.71	12.04	11.48	8.40	—
8	TBC BE	1.0	1.0	.10	.95	14	17	19.25	29.73	11.79	10.41	—
9	TBC BE	1.0	1.0	.18	.30	16	14	24.67	23.97	8.50	8.13	—
10	—	.2	.2	.00	.00	12	12	.20	.30	1.50	1.70	
11	—	.2	.2	.00	.00	14	15	.30	.59	1.58	1.69	
12	—	.3	.3	.00	.00	13	12	.50	.43	1.73	1.54	
13	—	.1	.1	.00	.00	12	14	.67	1.75	1.83	1.75	
14	—	.2	.2	.00	.00	16	17	.60	.43	1.63	1.73	
15	—	.3	.2	.00	.00	18	16	.30	.60	1.58	1.78	
16	—	.2	.2	.00	.00	15	14	1.83	1.55	1.42	1.33	
17	—	.3	.3	.00	.00	14	14	.85	1.32	1.66	1.68	
18	—	.2	.2	.00	.00	15	16	.55	.78	1.88	1.53	

ALT = argon laser trabeculoplasty; TBC = trabeculectomy; SLT = selective laser trabeculoplasty; BCVA = best-corrected visual acuity; LogMAR = logarithm of the minimum angle of resolution; IOP = intraocular pressure; HVF = Humphrey visual field; MD = mean deviation; PSD = pattern standard deviation; RE = right eye; LE = left eye; BE = both eyes.

TABLE 3. Main outcomes from the sleep questionnaire and sleep diary in control subjects and glaucomatous patients

	No. awakenings during the night (<3 episodes)	Sleep episodes during the day	Complaints about poor sleep quality	Work out for ≥20 min	Consume caffeinated beverage 1 or 2 times/d	Consume alcoholic drinks 1/d	Sleep interruption for noises or going to bathroom	Activity performed 1 h before going to sleep
Control subjects (n = 9)	8	4	1	5	8	2	8	Watch TV (6); read (3)
Glaucoma patients (n = 9)	9	3	6	4	7	3	9	Watch TV (6); read (2); use of computer (1)

Shown is the number of subjects referring to the different variables. Based on the sleep questionnaire and the sleep diary, no evident differences were found between groups, with the exception of the number of persons who complained about poor sleep quality.

of timolol and dorzolamide when needed to keep the IOP target.

No evident differences were found between groups based on the sleep questionnaire and sleep diary, particularly in relationship to the consumption of caffeinated/alcoholic drinks, physical activity, type (reading or watching TV) and duration of activity 1 h before going to sleep, wake-up times during sleep, reasons for waking at night (noise, going to the bathroom), and number of daytime naps, as shown in Table 3. Nevertheless, more patients with glaucoma (6/9) than control subjects (1/9) complained about poor sleep quality (Table 3). Figure 1 shows representative actograms and activity waveforms from control subjects (11, 12, and 16) and glaucomatous patients (1, 5, and 9). No significant differences were observed in the mean (± SEM) values of, respectively, the control and glaucoma groups for the activity rhythm period (1441 ± 2 vs. 1441 ± 3 min), amplitude (1647 ± 48 vs. 1449 ± 109 counts), MESOR (2271 ± 105 vs. 2442 ± 80 counts), and acrophase (15:29 h ± 27 min vs. 15:11 h ± 25 min). During the night, marginal locomotor activity was observed in control subjects, whereas glaucomatous patients were significantly more active (9.9% ± .9% vs. 15% ± 1.6% of the 24-h activity, for control and glaucoma groups, respectively; $p < .05$ by Student's t test). Statistical analysis of actigraphy data recorded during the 20 d indicated significant decrease in sleep minutes and sleep efficiency in glaucoma patients (Table 4). To further analyze activity rhythms, data from the Up interval (wakefulness state; Table 5) and Down interval (sleep state; Table 6) were analyzed separately. No significant differences were observed between control and glaucomatous

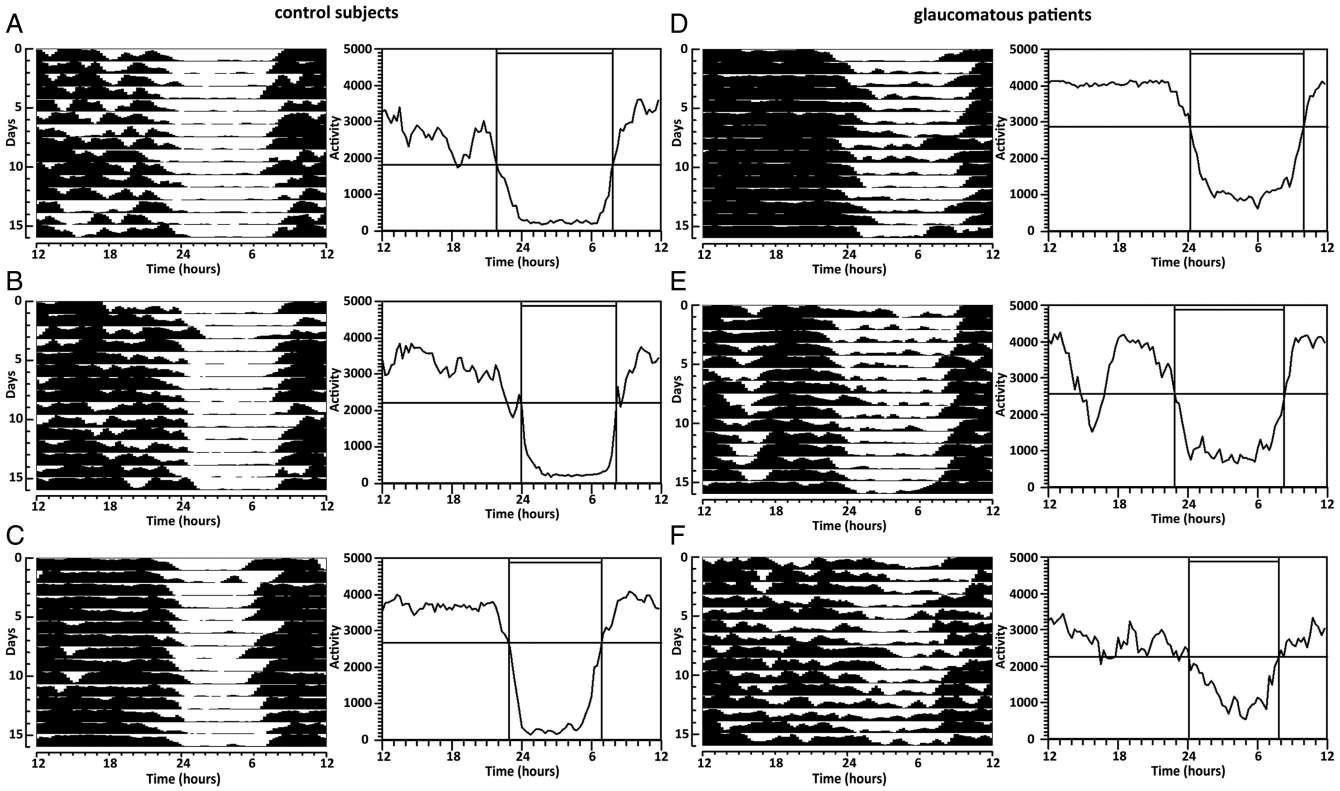

FIGURE 1. Representative actograms and waveforms from glaucomatous patients (D, E, and F: patients 1, 5, and 9, respectively) and control subjects (A, B, and C: patients 11, 12, and 16, respectively). Actograms: The horizontal axis shows the activity along the 24-h cycle, whereas the vertical axis shows consecutive cycles of activity. The black zone represents the wakefulness state, and the white zone indicates the sleep state. Waveform shows the average activity along the 24-h cycle for each subject. Vertical lines indicate average bedtime and wake-up time, indicated by each subject, whereas horizontal line represents the activity MESOR of each individual. Note the higher activity during the sleep state in glaucomatous patients.

TABLE 4. Statistical analysis of actigraphy data from control subjects and glaucomatous patients over a 24-h period

	Control		Glaucoma		
	Mean ± SEM	n	Mean ± SEM	n	
Number of intervals	18 ± 1	9	19 ± 1	9	ns
Duration (min) of the interval	1439.94 ± .01	9	1439.94 ± .01	9	ns
Mean activity score (arbitrary units)	153.55 ± 6.31	9	162.51 ± 5.42	9	ns
Wake minutes	959.36 ± 27.86	9	1071.97 ± 30.98	9	*
Sleep minutes	480.58 ± 27.86	9	367.97 ± 30.98	9	*
Sleep efficiency (%)	79.76 ± 4.03	9	59.05 ± 4.50	9	**
Sleep latency (min)	588.52 ± 44.34	9	526.6 ± 61.18	9	ns
Wake episodes	14.34 ± 2.49	9	18.77 ± 1.33	9	ns
Long wake episodes	5.35 ± .86	9	7.29 ± .90	9	ns
Sleep episodes	13.41 ± 2.48	9	17.77 ± 1.33	9	ns
Long sleep episodes	6.65 ± .79	9	8.59 ± .66	9	ns

Shown are mean ± SEM of parameters obtained from continuous actigraphic measurements for 20 d in control subjects and glaucomatous patients. ns = nonsignificant, *$p < .05$, **$p < .01$, Student's t test.

Wake minutes = total minutes scored as wake in the interval; sleep minutes = total minutes scored as sleep in the interval; sleep efficiency = 100 × sleep minutes/Down interval duration; sleep latency = minutes to start of first 20-min block with >19 min of sleep; wake episodes = number of blocks of contiguous wake epochs; sleep episodes = number of blocks of contiguous sleep epochs; long wake episode = wake episodes ≥10 min; long sleep episode = sleep episodes ≥10 min.

patients in daytime napping (i.e., sleep minutes: 44.6 ± 14.8 vs. 51.2 ± 11.7, or long sleep episodes: 1.4 ± .5 vs. 1.52 ± .39). In contrast, significant differences were observed during the Down interval, as shown in Table 6. During the night, glaucomatous patients had significantly more and longer waking episodes than controls, and a significant decrease in sleep efficiency.

No associations were detected between sleep-related disturbances by sex of subject, and, in addition, no obvious correlation was found between actigraphy and

TABLE 5. Statistical analysis of actigraphy data from control subjects and glaucomatous patients during the Up (wake) interval

	Control		Glaucoma		
Up interval	Mean ± SEM	n	Mean ± SEM	n	
Number of intervals	17 ± 1	9	18 ± 1	9	ns
Duration (min) of the interval	964.67 ± 13.94	9	1018.04 ± 20.93	9	*
Mean activity score (arbitrary units)	220.89 ± 7.2	9	218.89 ± 9.49	9	ns
Wake minutes	920.06 ± 26.2	9	966.82 ± 21.08	9	ns
Sleep minutes	44.61 ± 14.79	9	51.22 ± 11.73	9	ns
Wake episodes	6.19 ± 1.92	9	5.51 ± 1.21	9	ns
Long wake episodes	3.68 ± .85	9	3.54 ± .75	9	ns
Sleep episodes	5.24 ± 1.95	9	4.51 ± 1.21	9	ns
Long sleep episodes	1.4 ± .51	9	1.52 ± .39	9	ns

Shown are mean ± SEM of parameters in the Up interval obtained from actigraphic measurements of subjects for 20 d. ns = nonsignificant, * $p < .05$, ** $p < .01$, Student's t test.

TABLE 6. Statistical analysis of actigraphy data from control subjects and glaucomatous patients during the Down (sleep) interval

	Control		Glaucoma		
Down interval	Mean ± SEM	n	Mean ± SEM	n	
Number of intervals	18 ± 1	9	19 ± 1	9	ns
Duration (min) of interval	475.31 ± 13.18	9	420.51 ± 20.80	9	*
Mean activity score (arbitrary units)	15.03 ± 1.53	9	29.73 ± 4.26	9	**
Wake minutes	40.1 ± 5.33	9	103.05 ± 20.78	9	**
Sleep minutes	435.21 ± 15.60	9	317.46 ± 28.75	9	**
Sleep efficiency (%)	94.34 ± 1.07	9	79.31 ± 4.54	9	**
Sleep latency (min)	14.48 ± 2.22	9	25.86 ± 5.41	9	ns
Wake episodes	8.96 ± .85	9	14.34 ± 1.35	9	**
Long wake episodes	.89 ± .21	9	3.1 ± .58	9	**
Sleep episodes	8.01 ± .87	9	13.34 ± 1.35	9	**
Long sleep episodes	5.17 ± .46	9	7.09 ± .54	9	*

Shown are mean ± SEM of parameters in the Down interval obtained from actigraphic measurements for 20 d in control subjects and glaucomatous patients. ns = nonsignificant, *$p < .05$, **$p < .01$, Student's t test.

self-reported diurnal physical activity or consumption of caffeinated/alcoholic drinks.

DISCUSSION

The results of the present pilot study indicate significant changes in the sleep pattern of advanced glaucoma patients. Glaucoma patients showed more awakenings at night, shorter sleep duration, and lower sleep efficiency as compared with control subjects. Although no significant changes were found for circadian parameters, such as period, amplitude, or acrophase, of the locomotor circadian activity rhythm, the general pattern of actimetry from which sleep parameters were estimated indicated alterations in its robustness, suggesting a disrupted sleep-wake cycle. However, a note of caution should be taken regarding the analysis of locomotor activity rhythms used to approximate the 24-h activity pattern of human activity. We used Cosinor analysis, which fits the data to a cosine wave (in this case fit of a single 24-h waveform). Since locomotor activity usually exhibits a pattern that approximates a square, rather than a cosine, waveform, the amplitude and acrophase estimates can only be considered as preliminary representations for the data. Notwithstanding, disruption of the rest-activity pattern is very evident both by visual inspection of the data and by the analysis performed.

These alterations suggest decrease of quality-of-life parameters in glaucoma patients, since the quality and maintenance of the sleep-wake cycle represents a vital variable that affects most body functions. Indeed, some of these changes resemble age-related circadian alterations, since the aging process is associated with well-documented changes in sleep and seem to be associated with mortality risk (Paudel et al., 2010, 2011). Phase advance of circadian rhythms, in particular, of the sleep-wake cycle, and shortening of circadian period are noted in the elderly (Carrier et al., 2002). Moreover, decline in the amplitude of circadian rhythms, such as core body temperature, melatonin, sleep/wake, and cortisol secretion, has been reported in the elderly (Huang et al., 2002; Hofman & Swaab, 2006). In addition, significant loss of mRGCs with aging was recently described (La Morgia et al., 2011). However, although the influence of age-related factors cannot be completely ruled out, the present results cannot be merely attributed to aging, since age-matched subjects without glaucoma were

included in the control group. Some of the glaucomatous patients included in the present study (patients 1, 2, 3, 4, and 6) were medicated with topical antihypertensive drops. Therefore, the possibility that medications affected the circadian rhythms cannot be completely excluded. However, since different surgical or pharmacological treatments were used in the population of glaucoma patients included in this study, it seems unlikely that sleep rhythm alterations could be merely attributable to antihypertensive medication. Normal sleep circadian patterns are fundamental for healthy conditions (Pandi-Perumal et al., 2006). The diagnosis of sleep disorders typically involves sophisticated procedures and equipment that are intrusive to the patient. Wrist actigraphy, on the contrary, is a noninvasive and low-cost solution to gather data that can provide valuable information in the diagnosis of these disorders, due to its ability to register behavioral data under normal life conditions (Cole et al., 1992; Jean-Louis et al., 2001; Paquet et al., 2007). The acquired data may be used to infer the sleep/wakefulness state of the patient during the circadian cycle and to detect abnormal behavioral patterns (Domingues et al., 2010). Along the circadian cycle, a different pattern of movements occurs during wakefulness and sleep (Lötjönen et al., 2003). During the day (typically wakefulness state) movements are usually very heterogeneous and dense, whereas during the night (sleep state) movements are more sparse. In order to more specifically analyze the effect of glaucoma on sleep quality, activity patterns during sleep and wake phases were separately analyzed. As shown herein, clear differences between groups were observed in the sleep, but not in the wakefulness, state, which supports the hypothesis that circadian misalignment in glaucomatous patients could be attributed to sleep disturbances. In fact, even a qualitative appreciation of the actograms showed clear differences between the wakefulness and sleep states in control subjects, whereas in glaucomatous patients, more activity during the sleep state was evident. Notably, a similar profile was observed in rats with experimental glaucoma induced by CS, showing a significant increase in the percentage of locomotor activity in the photophase (the rest phase in nocturnal animals), as compared with control rats (de Zavalía et al., 2011).

Blind human subjects have always been considered a useful model for the study of circadian rhythm disorders (Sack & Lewy, 2001). However, in most studies, enrolled subjects were affected by heterogeneous retinal pathologies, and the occurrence of circadian rhythms misalignment was not considered with respect to the specific type of retinal disease. Based on the available diverse data, the occurrence of circadian photoentraiment disturbances seemed to be determined by the absence of light perception. In that sense, the vast majority (77%) of blind subjects with light perception have normally entrained circadian rhythms, suggesting preservation of the mRGC-SCN circuit. In contrast, most of the subjects without light perception, especially those with ocular enucleation, have abnormal circadian photoentrainment (Skene & Arendt, 2007). Thus, circadian rhythm disturbances seem to be related to the severity, as well as type, of ocular disease (Wee & Van Gelder, 2004). In that context, some studies have suggested that visually impaired subjects are at risk for both self-reported (Tabandeh et al., 1998) and objectively measured (Stores & Ramchandani, 1999) sleep disorders. These sleep disorders are thought to arise, at least in part, from the inability of the circadian clock to entrain correctly to external light and dark signals in visually impaired individuals (Klerman et al., 1998). Abnormally high levels of daytime napping, increased wake-up time instability, and increased sleep latency, as measured by wrist actigraphy, were previously demonstrated in a subset of visually impaired children and young adults, and it was suggested that the nature of eye disease—specifically, whether the optic nerve is or is not the primary cause of disease—in large part determines the probability of pathologic levels of daytime sleepiness, as measured by daytime napping (Wee & Van Gelder, 2004). It should be noted that a heterogeneous group of optic nerve diseases, such as end-stage glaucoma, retinopathy of prematurity with long-standing bilateral stage 5 disease, optic nerve hypoplasia, and bilateral optic nerve trauma, were included in this study. Evidently, since all these diseases lead to visual impairment and even blindness, they indirectly provoke relative physical inactivity, which may cascade into sleep problems and daytime sleepiness. Likewise, afflicted individuals would have less opportunity for bright-light exposure, which may cause circadian rhythm dysfunctions (Kripke, 1998). In the present study, only glaucomatous patients were included, and no significant differences in daytime naps were observed as compared with control subjects. In contrast to the aforementioned ocular diseases, the effects of glaucoma on the circadian timing system might be twofold: (i) direct impact through degeneration of RGCs and, eventually, also of mRGCs; and (ii) indirect impact through social isolation due to blindness, as is the case for other ophthalmic diseases (Wee & Van Gelder, 2004).

Although several groups have examined the involvement of mRGCs or melanopsin in experimental glaucoma in rodents (de Zavalía et al., 2011; Drouyer et al., 2008; Jakobs et al., 2005; Li et al., 2006; Wang et al., 2008), to date, few studies investigated the mRGC system directly in human glaucoma (Kankipati et al., 2011; Pérez-Rico et al., 2010). In that sense, disturbance of the autonomic nervous system circadian rhythm was shown in patients with glaucoma (Kashiwagi et al., 2000). It was demonstrated that the light-induced suppression of melatonin secretion is substantially unaffected in other neurodegenerative optic neuropathies, such as Leber hereditary optic neuropathy and dominant optic atrophy (La Morgia et al., 2011), in which the pupil light reflex is also preserved (Bremner et al., 2001; Wakakura & Yokoe, 1995). Thus, it is tempting to speculate that

mRGCs are particularly susceptible to glaucomatous damage, and that glaucoma is a key model that needs to be further investigated in regards to circadian photoreception in optic neuropathies.

In summary, although these findings should be replicated in large-scale studies before definitive conclusions can be reached, these results are consistent with sleep alterations in advanced glaucoma, suggesting attention should be paid to non–image-forming visual functions, such as control of circadian rhythms and its clinical impact in patients with glaucoma, even when most of them retains some residual visual function. Glaucoma may affect the quality of life in several ways. These include the visual effects of the disease itself (decreased visual field), psychological effects of diagnosis (specifically fear of blindness), potential side effects of treatment (either medical or surgical), and financial effects (cost of visits and therapy). The present results suggest another risk to the quality of life of patients with glaucoma, i.e., alterations of circadian physiology. Circadian rhythm disorders may include poor concentration, sleep problems, impaired performance, decrease in cognitive skills, poor psychomotor coordination, and headaches, among many others. There are several therapeutic strategies to restore circadian balance. Identifying and treating circadian disorders in glaucoma will help to improve the quality of life of patients with this ocular disease.

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