

Title: Access to electric light is associated with delays of the dim light melatonin onset in a traditionally hunter-gatherer Toba/Qom community

Running title: Electric light and a delayed clock in the Toba/Qom

Leandro P. Casiraghi^{1,*}, Santiago A. Plano^{2,3,*}, Eduardo Fernández-Duque⁴, Claudia Valeggia⁵, Diego A. Golombek³ and Horacio O. de la Iglesia¹

¹Department of Biology, University of Washington, Seattle, Washington, USA

²Institute for Biomedical Research (BIOMED), Catholic University of Argentina/ CONICET, Buenos Aires, Argentina.

³Universidad Nacional de Quilmes/CONICET, Buenos Aires, Argentina

⁴Department of Anthropology and School of Forestry and Environmental Studies, Yale University, New Haven, Connecticut, USA

⁵Yale University, New Haven, Connecticut, USA

*Authors contributed equally

Correspondence:

Diego A. Golombek

Departamento de Ciencia y Tecnología

Universidad Nacional de Quilmes

Roque Sáenz Peña 352

(1876) Bernal - Buenos Aires, Argentina

Email: dgolombek@unq.edu.ar

Horacio O. de la Iglesia

Department of Biology

Life Science Center

University of Washington

Seattle, WA 98195-1800

Email: horacioid@uw.edu

Abstract

Key to the transition of humans from nomadic hunting-gathering groups to industrialized and highly urbanized societies was the creation of protected and artificially lit environments that extended the natural daylight hours and consolidated sleep away from nocturnal threats. These conditions isolated humans from the natural regulators of sleep and exposed them higher levels of light during the evening, which are associated with a later sleep onset. Here we investigated the extent to which this delayed timing of sleep is due to a delayed circadian system. We studied two communities of Toba/Qom Argentina, one with and the other without access to electricity. These communities have recently transitioned from a hunting-gathering subsistence to mixed subsistence systems and represent a unique model in which to study the potential effects of the access to artificial light on sleep physiology. We have previously shown that participants in the community with access to electricity had, compared to participants in the community without electricity, later sleep onsets and shorter sleep bouts. Here we show they also have a delayed dim light melatonin onset (DLMO). This difference is present during the winter but not during the spring when the influence of evening artificial light is likely less relevant. We also confirm that the difference in DLMO cannot be accounted by differences in clock gene polymorphisms. Our results support the notion that the human transition into artificially lit environments had a major impact on physiological systems that regulate sleep timing, including the phase of the master circadian clock.

Introduction

Humans typically display a sleep-wake cycle characterized by diurnal activity and nocturnal sleep. This pattern of rest-activity was likely adaptive for our hunter-gatherer ancestors allowing them to exploit a highly developed visual system to seek food, interact with conspecifics and avoid predators, as well as to engage in the risky behavior of sleeping during the night within protected environments¹. Even when these selective pressures are not present in modern society, a vast majority of humans maintain this pattern of diurnal activity and a consolidated bout of nocturnal sleep.

The daily timing of sleep results from the regulation by two processes. The first one is a homeostatic response by which extended wakefulness continuously increases the need to sleep until the sleep pressure is high enough to trigger sleep onset². The second process is the result of the activity of a master circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus^{3,4}. Through input to both sleep and wake centers, the SCN determines the timing of sleep and wake; this timing is, in principle, independent of the previous wake time. The sleep-wake cycle remains synchronized to the 24-h environment by means of direct retinal projections to the SCN, which entrain the clock to the light-dark (LD) cycle⁵. The two regulatory branches interact to generate a daily nocturnal bout of sleep. Under photoperiods that are not extremely asymmetrical and when the only source of light is the natural daylight, entrainment leads to a sleep timing with an onset that follows sunset by a couple of hours and an offset that coincides with, or precedes sunrise.

We confirmed this expected timing of sleep under natural daylight in a community of the Toba/Qom people from the Argentinean Chaco in a previous study⁶. Using wrist activity monitors, we showed that participants living in rural areas without access to electric light had nocturnal bouts of sleep that lasted slightly over 8 hours, had sleep onsets that coincided with astronomical dusk (the true onset of darkness after sunset), and sleep offsets that coincided with sunrise. The Toba/Qom participants live in a remote area of the Formosa province in northern Argentina and they still rely, to some extent, on hunting and gathering for subsistence⁷⁻⁹. Thus, we consider the regulation of their sleep as a proxy for how sleep was ancestrally regulated in humans. Indeed, this timing of sleep under natural daylight is hardly found in modern human societies. Although most modern humans continue to wake up around the time of sunrise, particularly during working days, their bedtimes have been substantially delayed given their ability to artificially extend daylight^{10,11}. We found that this was also the case in a Toba/Qom community that do have 24/7 access to electricity and live 50 km away from those without access to electricity. Participants in this community had sleep bouts that started later in the evening but still ended at sunrise, leading to a shorter daily sleep bout than the bouts of participants without electricity⁶.

Our findings are in line with previous research showing that access to electricity in more urban communities lead to later bedtimes and, in some cases, shorter daily sleep¹²⁻¹⁵. This later sleep timing could be the result of the direct effects of light that inhibit sleep¹⁶ and melatonin release, which marks the start of the biological night¹⁷; affecting the homeostatic buildup of sleep pressure. On the other hand, the later sleep

timing could also be the result of a delay in the master circadian clock by exposure to artificial light during the evening^{18,19}. The timing of sleep alone does not provide an accurate assessment of the phase of the circadian pacemaker; the standard to determine the clock's phase is the evening onset in the release of melatonin under dim light conditions²⁰.

In the present study we sought to evaluate **the hypothesis that a change in the phase of the circadian pacemaker is involved in the delayed sleep timing observed when humans gain access to electricity. According to this hypothesis,** we expect that the dim-light melatonin onset (DLMO) would be delayed in the community with access to electricity compared to the community without access to electricity, and that the delay would be smaller at a time of the year when the natural photoperiod is longer and the influence of evening electric light more negligible.

Materials and Methods

Protocols and Ethics

All the procedures were approved by the Internal Review Board of the Human Subjects Division at the University of Washington (Seattle, USA) and are in accordance with the declaration of Helsinki. All participants gave verbal consent (**approved by the Review Board in place of written consent**) to participate after being informed, in Spanish, of all procedures by an investigator. For participants under 18 years of age, we requested their consent and the consent from one of their parents.

Participants

Toba/Qom participants belonged to one of two Western Toba/Qom communities in the province of Formosa, northern Argentina, that share the same ethnic and historical past:

1) An urban community (*Electricity*) located in the outskirts of Ingeniero Juárez (23°47' S, 61°48' W), a town with 10,000 inhabitants. All the participants in this community had 24-hour access to electric light at home, and outside through streetlight poles. This community originated from a group of Toba/Qom people who migrated from a northern region in the 1990s^{7,8}.

2) A rural community (*No-electricity*) located near Vaca Perdida (23°29'S, 61°38' W); it is a small rural settlement of approximately 300 people 50 km north of Ingeniero Juárez. These participants had no access to electric light.

All non-school age participants in our sample were unemployed, and they typically show no difference in sleep timing between weekdays and weekends. Nevertheless, all saliva samples (see below) were taken on an evening before a weekday.

Melatonin determinations

Sixty-two participants participated in the study (Electricity = 30, No-electricity = 32). Eight to nine hourly saliva samples (**see Table 1 for sampling dates and times**) were taken from each participant, with the last sample taken **at least** one hour after the

average bedtime for each community⁶. Participants in the No-electricity community were asked to stay outdoors while the samples were taken, as there were no indoor environments where all the participants could gather. For the winter sampling, participants in the Electricity community were asked to move into a dim-light room after the afternoon daylight intensity was below 1000 lux; the last five samples were all taken under a light intensity below 10 lux. For the spring sampling, participants in the Electricity community were asked to stay in a group outdoors, far from any source of artificial light; the last six samples were taken under a light intensity below 10 lux. The reason for this difference between winter and spring sampling is that during the winter sampling the ambient temperature was very low whereas in the spring temperatures are typically 30-40°C, making it uncomfortable and even unhealthy to remain inside rooms without any refrigeration system. Participants were socializing with each other during the sampling, were standing while samples were taken, and at all times were asked not to engage in physical activity. They were provided with food and water between samples, but were asked not to drink or eat anything 20 min before each sample. Participants were asked whether they had had a good night of sleep the night before, and if they had taken any naps during the day of the sampling. Samples from participants who declared having had little sleep the night before, or that they had slept a long nap that day, were later discarded. Saliva samples were stored immediately in wet ice, and frozen within 48 h for later analysis. After collection, samples were shipped to the Universidad Nacional de Quilmes in Buenos Aires Province, where melatonin concentration was determined in duplicates of each sample using the BÜHLMANN Direct Saliva Melatonin ELISA kit (Bühlmann Laboratories, Schönenbuch, Switzerland) following the manufacturer's instructions. The lower limit of detection was considered to be 0.5 pg/ml, as indicated by the manufacturer, and samples that were measured below this threshold were considered to be at such threshold value. The inter-assay variability was estimated at 28%, a little over the 23% declared by the manufacturer.

The DLMO for each participant was determined according to the method described by Voultsios et al.²¹. First, we determined the mean and the standard deviation of the concentration of melatonin in the baseline condition, and determined the concentration of the relative threshold of the onset according to the formula:

$$\text{Mel}_{(\text{onset})} = \text{mean}[\text{Mel}_{(\text{baseline})}] + 2 * \text{SD}[\text{Mel}_{(\text{baseline})}]$$

Then, we linearly extrapolated the time at which this concentration would have been measured within the first interval of determinations in which this concentration was contained. The clock time of the calculated onset was relativized to the time of solar dusk on the day of sampling. We randomly chose five participants from those that presented good quality samples and replicated the protocol to test the variability of the determination. The mean difference in the calculated onset of melatonin release from replicate assays was 25 min.

Six participants did not comply with the sample collection protocol and their samples were discarded. Samples from 10 participants were later discarded because they were not of sufficient quality to be able to determine the melatonin onset using the method above. Onset data from seven participants were discarded after their calculated onsets were considered as outliers within their groups through a median absolute deviation (MAD) approach with a threshold of 3 MADs, using the *Routliers* package

from R²². The final number of participants for each season and community is shown in Table 1.

Statistical analysis

All statistical analysis and plotting were performed using R on the RStudio software²³. To evaluate the strength of the association between the availability of electric light and season on melatonin onset, we fitted a Linear Model (LM) including sex and age, season, community, and the interaction of season and community as fixed factors, using the *lme4* package²⁴. Partial Eta-Squared values are indicated as measures of effect size and were calculated with the *effectsize* package²⁵. Tukey multiple comparisons after the LM fit were performed with the *multcomp* package²⁶. Linearity of the model residuals was verified through visual inspection of a QQ-plot of the normalized residuals. All plots were performed with the *ggplot2* package²⁷.

Results

Times of DLMO for the two communities in both seasons are summarized in Table 2 and shown in Fig. 1. Season and community factors interacted in a relevant manner to explain the timing of the melatonin onset (part-eta² = 0.30, $p = 0.0007$; Table 3). In the Electricity community the DLMO occurred, on average, 72 minutes later during the winter than the spring (estimated differences calculated from the linear model; 95% CI: 33/111; Tukey $p = 0.0001$), and 61 minutes later than in the No-Electricity group in the winter (95% CI: 20/102; $p = 0.0022$). Negligible differences were detected between the communities during the spring season (11 minutes earlier in the Electricity community; 95% CI: 43/-18; $p = 0.6806$). No differences between seasons for the No-Electricity community were detected (95% CI: -33/31; $p = 0.9998$).

Discussion

Our data show that the winter circadian phase of Toba/Qom people during living without access to electricity is considerably advanced compared to people who belong to the same ethnic group, and share the same sociocultural background, but have free access to electricity. Whereas the winter is characterized by a later DLMO in the electricity community (71 minutes), this difference is not present in the no-electricity community, clearly pointing to a potential effect of evening exposure to artificial light, which delays the phase of the circadian system. Similarly, whereas a clear difference in circadian phase between communities was present in the winter, this difference was not evident under the longer natural photoperiod of the spring. These results are in line with our previous study that showed a later onset and shorter duration of sleep in the electricity community, and that this difference was larger in the winter⁶. They also suggest that sleep timing differences between communities under longer natural photoperiod may not rely on changes in circadian phase. Together, the present results indicate that the difference in sleep timing between the electricity and no-electricity community arises, at least in part, from phase differences in the master circadian pacemaker. They also show that differences in sleep timing during the winter can be

attributed to differences in light exposure between communities, and not necessarily to social correlates of the more urban life to which the Electricity community is exposed.

A delayed circadian phase in association with exposure to artificial light is expected based on previous laboratory studies^{18,28}. However, few studies have addressed the extent to which the differential access to electricity under field conditions affects circadian phase. Peixoto et al. addressed this question by comparing adolescents with and without access to electricity but while they demonstrated an effect of school schedule they failed to demonstrate an effect of the access to electricity²⁹. In contrast, a study on Amazon rubber tappers showed an effect of access to electricity on the DLMO¹². Importantly, the Toba/Qom communities we studied represent a unique scenario in which the effect of access to electric light can be studied for several reasons. These communities used to rely solely on hunting-gathering only two-to-three generations ago, and their transition into other forms of subsistence, and into environments that offer the opportunity to sleep in more protected and artificially lit environments, represents a proxy for the transition humans experienced from hunting-gathering groups to highly urbanized environments³⁰. Second, these communities are socioculturally homogeneous, preventing confounds that emerge from differences in daily activities. Third, participants across communities share a common ethnic background, minimizing potential genetic differences between communities^{7,8}. As an example, a screening for the frequency in the 5-repeats allele of the VNTR polymorphism in *hper3*, which has been linked to both the timing of the DLMO and diurnal preference^{31,32}, revealed a null variability among 70 Toba/Qom participants from both communities, with every single participant being homozygous for the 4-repeat allele. This result shows a striking homogeneity compared to previous studies, including one with control participants in Argentina^{33,34}. Although future studies should further explore the genetic basis of sleep timing and circadian phase in the Toba/Qom people, the low variability we found in this locus supports the notion that these communities are highly homogeneous genetically, and minimizes the chance that the differences we found between communities are due to genetic differences.

While the DLMO assessment is considered the best test for the determination of the phase of the circadian clock in humans, it is usually applied under very controlled conditions. Our study is limited by the number of participants it includes, and because saliva samples were taken under very fragile field conditions, as these Toba/Qom communities live in one of the most remote and underdeveloped areas in Argentina. Some communities can only be reached through very rugged roads, and we have very limited possibilities to sample under more controlled conditions and from many participants. The samples taken under natural light conditions could have potentially masked differences between communities in the DLMO during the spring season. We do not think this is the case because both communities show a DLMO that occurs just after dusk, and a later DLMO in the Electricity community would have been revealed under our sampling conditions. Despite these limitations, our study is unique in investigating ethnically homogenous communities living in contrasting light environments.

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Figure Legends

Figure 1. A) Dim light melatonin onset (DLMO) from the time of dusk in the two communities measured in spring and winter, shown as boxplots along with each participant value. Brackets indicate p-values (corrected for multiple comparisons) from Tukey comparisons after the Linear Model fit. Participants per community and season: Electricity, Winter = 6; Electricity, Spring = 9; No electricity, Winter = 7; No electricity, Spring = 17. **B)** Example patterns from a representative participant in each community in each season. The curves describe the evolution of the ratio of the melatonin concentration to that expected at the onset in function of the time from solar dusk. The dashed horizontal line indicates the concentration ratio expected at the onset of melatonin release.

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Tables

Table 1. Dates and time of saliva samplings

Community	Date (n)	Season	Sunset time	Photoperiod length	Time of first sample (# of samples)
No-Electricity	Oct 16, 2016 (25)	Spring	19:18	12h53min	14:00 (9)
	Aug 6, 2014 (7)	Winter	18:46	11h10min	15:00 (9)
Electricity	Nov 9, 2017 (15)*	Spring	19:26	13h10min	16:00 (8)
	Nov 14, 2018 (10)*	Spring	19:29	13h15min	17:00 (9)
	Aug 7, 2014 (7)	Winter	18:46	11h10min	16:00 (8)

Note: the *n* values indicated represent the total participants sampled on each date, including those later discarded as described in the Methods. * Two subjects were sampled twice, once on each of these dates. For one of these we were able to measure the DLMO from both samplings; the values were averaged for the analysis.

Table 2. Data summary

Community	Season	n	DLMO (min from dusk)		
			Mean	SD	95%CI
No-Electricity	Spring	17	52	24	39/64
	Winter	7	51	39	14/87
Electricity	Spring	9	39	9	33/46
	Winter	6	104	36	66/142

Table 3. Linear model analysis coefficients

Factor	LM Estimates (delta min)			ANOVA			Effect size	
	Mean	95%CI	S.E.	df	F	p	part-eta ²	90%CI
(Intercept)	65	39/90	13	-	-	-	-	-
Sex - Male	-10	-29/9	9	1	0.0055	0.9412	0.00	0.00/0.01
Age	-0.40/yr	-1/1	0.50	1	0.5817	0.4510	0.02	0.00/0.15
Season - Winter	-1	-26/24	12	1	8.7909	0.0056	0.21	0.04/0.40
Community - ELEC	-12	-36/12	12	1	1.9980	0.1669	0.06	0.00/0.22
Comm.XSeason	73	33/113	20	1	13.9778	0.0007	0.30	0.10/0.48

