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Conflicted clocks: Social jetlag, entrainment and the role of chronotype

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Chapter 8

Melatonin expression: winter and summer, week in and week out

Giulia Zerbini, Till Roenneberg, and Martha Merrow

Abstract

Living organisms have developed an internal time-keeping mechanism (circadian clock) to adapt to the regular 24-hour changes in the environment. Entrainment is the process that keeps the circadian clock synchronized with a stable phase relationship with its zeitgeber (external time cue). In humans, the most important zeitgeber for the timing of behavior is light. There is a dearth of studies investigating entrainment in real life conditions, taking into account individual differences in phase of entrainment (chronotype) and varying light environments. Here, we aimed to investigate the influence of season (summer vs. winter) and weekly schedule (workdays vs. work-free days) on phase of entrainment (assessed via dim-light melatonin onset; DLMO). We collected data about sleep and activity in 33 participants for 10 days at approximately the 21st of June (longest photoperiod) and the 21st of December (shortest photoperiod). In addition, we assessed DLMO on a workday and on a work-free day, both in summer and in winter. We did not find any clear influence of season on the parameters assessed. In contrast, all parameters varied according to the weekly schedule. Sleep and activity were later on work-free days. A chronotype-dependent influence of daily activities or schedule on DLMO was found, with late chronotypes showing a later DLMO on work-free days. Morning light (between 6:00 h and 12:00 h) was the strongest predictor for the variation in DLMO, with increased exposure to morning light associated with an earlier DLMO. Late chronotypes were exposed to less and later morning light on work-free days relative to workdays in comparison with early chronotypes, possibly explaining the difference in DLMO between workdays and work-free days.

Our results show that the habitual weekly schedule of late chronotypes is able to phase-shift their phase of entrainment (assessed via DLMO) between workdays and work-free days. To counteract this delay in DLMO over the weekend, late chronotypes should increase their morning light exposure on work-free days.

Introduction

Organisms from bacteria to humans have evolved an internal time keeping mechanism (circadian clock) that regulates biological processes in synchrony with the 24-hour variation in environmental conditions (e.g. alternation between day and night). Entrainment is the process that keeps the circadian clock synchronized with a stable phase relationship to its zeitgeber (external time signal) (Aschoff, Klotter, & Wever, 1964). Phase of entrainment varies between individuals leading to a distribution of chronotypes, ranging from early to late (Roenneberg et al., 2007a). On one hand, chronotype is easily assessed with questionnaires to facilitate high throughput analysis of clock regulated behavior (e.g. Munich ChronoType Questionnaire (MCTQ); Roenneberg, Wirz-Justice, & Mellow, 2003; Morningness-Eveningness Questionnaire (MEQ); Horne & Ostberg, 1976). On the other hand, physiological markers (e.g. melatonin) are not so easy to obtain (expensive, biological samples to be harvested) yet they are considered a more reliable measure of phase of entrainment. Both chronotype assessed with MCTQ (midpoint of sleep on work-free days sleep corrected; MSF_{sc}) and with the MEQ correlate with dim-light melatonin onset (DLMO) (MCTQ: $r = 0.68$; MEQ: $r = -0.70$; Kantermann, Sung, & Burgess, 2015). DLMO is often the first choice because the melatonin rhythm is thought to be under the direct control of the circadian clock and is quite robust and stable (Arendt, 2006; Klerman, Gershengorn, Duffy, & Kronauer, 2002).

How does entrainment work? The most important zeitgeber for human entrainment is light, which enters the circadian system through the eyes (Duffy & Wright, 2005; Roenneberg & Foster, 1997; Roenneberg, Kumar, & Mellow, 2007b; K. P. Wright et al., 2013). Exposure to light at specific times of day can phase shift the clock in opposite directions. For instance, a light pulse at the beginning of the biological night (evening) delays phase of entrainment, whereas a light pulse at the end of the biological night (morning) advances it (Khalsa, Jewett, Cajochen, & Czeisler, 2003). Duration and intensity of a single light pulse influence the strength of the phase shift (St Hilaire et al., 2012; K. P. Wright, Hughes, Kronauer, Dijk, & Czeisler, 2001; Zeitzer, Dijk, Kronauer, Brown, & Czeisler, 2000). In addition, the circadian system was found to be more sensitive to the effects of light in the evening after being exposed to dim light compared to bright light during the day (Hebert, Martin, Lee, & Eastman, 2002). Indeed, the circadian clock does not only respond to single light pulses, but rather integrates any light detected during the day and consequently compresses/expands its cycle in order to keep a stable phase of entrainment (Roenneberg, Hut, Daan, & Mellow, 2010). All these studies have described the isolated effects of light on the circadian clock mainly in highly controlled laboratory conditions. However, entrainment is a very complex phenomenon that results from the integration of several external and internal time signals. For this reason, more field studies investigating entrainment in real life conditions are needed.

Seasonal changes in photoperiod (day length) give a unique opportunity to study how phase of entrainment (DLMO) may vary depending on differences in light exposure. Especially at high latitudes, photoperiod (day length) is longer in summer compared to winter (e.g. in Amsterdam, The Netherlands: summer photoperiod: 16:48 h and winter photoperiod: 7:40 h).

Based on laboratory studies, the symmetrical expansion of photoperiod in summer should not influence phase of entrainment because the advancing and delaying effects of both increased morning and evening light exposure would be canceled out. However, increased daily light exposure (stronger zeitgeber) has been associated with an earlier phase of entrainment, suggesting that in summer (more light) phase of entrainment could be earlier than in winter (Roenneberg & Merrow, 2007).

There are only a few studies that have assessed phase of entrainment (via DLMO or peak of the melatonin rhythm) across different seasons in the same individuals and they describe conflicting results. No difference in DLMO was found between summer and winter in a working population (Crowley, Molina, & Burgess, 2015). In adolescents, DLMO was shown to be later in spring relative to winter (Figueiro & Rea, 2010). When looking at peak melatonin, this was found to be earlier in summer relative to winter (K. Honma, Honma, Kohsaka, & Fukuda, 1992; Illnerová, Zvolnsky, & Vaněček, 1985). Finally, some studies have shown that the duration of melatonin secretion matches the length of photoperiod, but only in laboratory conditions (or when exposure to natural photoperiod was manipulated) and in natural (not electrical) lighting conditions (Stothard et al., 2017; Vondrasová-Jelínková, Hájek, & Illnerová, 1999; Wehr, 1991). These discrepant findings might derive from assessing the melatonin rhythm in different months within the same season, in different conditions (laboratory, manipulated photoperiod, electrical or natural lighting conditions), and from selecting relative small sample sizes (range in these studies: 6 - 16 participants).

It is also possible that a photoperiodic response in humans is reduced as a consequence of a 24-hour availability of artificial light, leading to little differences in perceived day length between summer and winter (Arendt, Middleton, Stone, & Skene, 1999).

Light exposure varies not only across seasons, but also between workdays and work-free days. Generally we are exposed to more morning light on workdays because of commuting to work (Crowley et al., 2015). There are also individual differences in light exposure, with early chronotypes usually being exposed to more morning light and less evening light (Goulet, Mongrain, Desrosiers, Paquet, & Dumont, 2007). Especially in late chronotypes, there is a clear difference between sleep timing on workdays and on work-free days, with the midpoint of sleep on work-free days being later. This phenomenon is known as social jetlag (Wittmann, Dinich, Merrow, & Roenneberg, 2006). Whether phase of entrainment (assessed via DLMO) also changes between workdays and work-free days is not clear yet. There are a few studies that have experimentally manipulated sleep timing or duration to simulate a typical weekend, resulting in a later DLMO following a later or longer sleep duration (Burgess & Eastman, 2006; Crowley & Carskadon, 2010; Jelínková-Vondrasová, Hájek, & Illnerová, 1999; Taylor, Wright, & Lack, 2008; Yang, Spielman, & Ambrosio, 2001)

Here, we aimed to better understand entrainment in real life conditions by looking at the influence of season (summer vs. winter) and weekly schedule (workdays vs. work-free days) on DLMO, sleep and activity. We assessed these parameters at approximately the 21st of June (shortest photoperiod) and the 21st of December (longest photoperiod) in the same individuals (N = 33) without any restriction/modification to their habitual life-style. In addition, we measured chronotype in order to control for the impact of light perceived at different phases on entrainment.

We found chronotype-specific response characteristics to the influence of weekly schedule on DLMO, with late chronotypes exhibiting a significant delay over the weekend. Morning light exposure was the strongest predictor of the variation in DLMO. Only in late chronotypes exposure to light in the morning was decreased and delayed on work-free days, possibly explaining the later DLMO after the weekend.

Methods

The study was conducted in June 2016 and in December 2016 in Groningen (53°13' N / 6°33' E), The Netherlands. Participants were recruited via online posts and flyers. Only participants with a regular weekly schedule (at least 4 workdays per week) were selected. Students were not allowed to participate in the study. Other selection criteria involved no shift work in the past 5 years and no travel across more than 2 time zones during the month before the study started. Females could participate only if they made use of hormonal contraceptives (to avoid possible fluctuations in melatonin levels and sleep quality depending on the phase of the menstrual cycle; Lee Barron, 2007). 35 participants (20 females; mean age 29 years \pm SD 4.9, age range: 22 - 40) completed the first part of the study in summer. In winter, there was one dropout and one participant changed work schedule to an irregular one and was therefore excluded from all analyses. The final sample size consisted of 33 participants (18 females; mean age 29 years \pm SD 4.9, age range: 23 - 40). The majority of the participants (N = 24) worked from Monday to Friday. The remaining 9 participants worked 4 days per week with a work-free day either on Wednesdays or on Fridays. The usual office hours in The Netherlands are from 9:00 h till 17:00 h.

The protocol lasted 10 days, starting on a Friday and ending the following week on a Sunday (Fig.1). The same protocol was run in summer (between 17.06.16 - 03.07.16) and in winter (between 02.12.16 - 18.12.16). Photoperiod (day length) was 16:58 h in summer (civil twilight at 4:14 h and at 22:57 h; sunrise at 5:06 h and sunset at 22:05 h) and 7:31 h in winter (civil twilight at 8:03 h and at 17:00 h; sunrise at 8:46 h and sunset at 16:17 h). Before the protocol started (both in summer and in winter), participants filled in the Munich ChronoType Questionnaire (MCTQ; Roenneberg et al., 2003), the Beck Depression Inventory (BDI; Beck, Steer, & Brown, 1996), and the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The MCTQ was used to assess chronotype as the midpoint of sleep on work-free days (MSF) corrected for sleep debt accumulated on workdays (MSF_{sc}). The BDI and PSQI were used to assess depressive symptoms and subjective sleep quality. During the protocol participants filled in a daily sleep diary and wore continuously an actiwatch (MotionWatch 8, CamNtech, Cambridge, UK). Actigraphy data were analyzed with ChronoSapiens (version 9).

On two evenings (on a workday and on work-free day) participants collected 7 hourly saliva samples at home, starting 5 hours before and finishing 1 hour after habitual sleep onset (weighted average sleep onset on workdays and on work-free days based on the participants' answers to the MCTQ). During the entire saliva sample collection participants stayed in dim

light, wore a pair of blue-light-blocking glasses, and were not allowed to use toothpaste and to consume coffee, tea, alcohol, chocolate, banana, and food with artificial additives. The saliva samples were collected using Salivettes (Sarstedt, Nümbrecht, DE). The samples were first frozen at -80°C , and later analyzed using direct saliva melatonin radioimmunoassay (RIA) test kits (Bühlmann, Schönenbuch, CH). The time of dim-light melatonin onset (DLMO) was calculated by linear interpolation between the time points before and after melatonin concentrations crossed and stayed above the threshold of 3 pg/mL . The lower limit detection of the kit was below 0.5 pg/mL . The intra-assay coefficient of variability was 13.33% (low melatonin) and 12.66% (high melatonin), while the inter-assay coefficient of variability was 9.76% (low melatonin) and 12.11% (high melatonin).

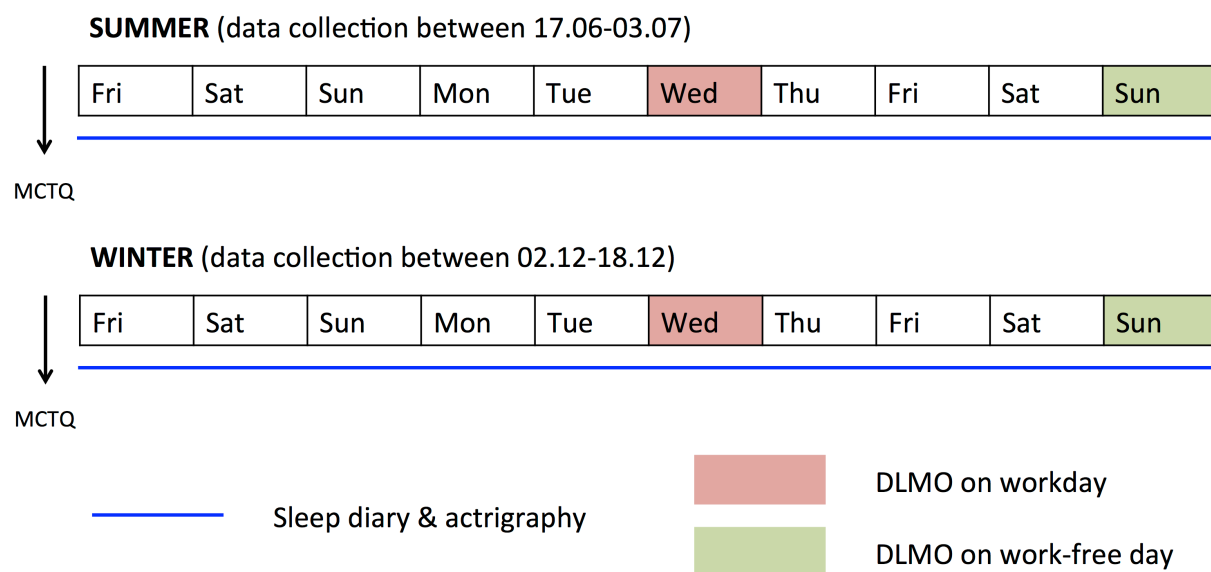


Figure 1. Experimental design.

Before each data collection, participants filled in the MCTQ. During the data collection, participants filled in a daily sleep diary and continuously wore an actiwatch (for light and activity data recording). On a workday (red) and on a work-free day (green), participants took 7 hourly saliva samples to assess DLMO.

Statistical analyses were done using R software (R version 3.3.0; The R Core team, 2013). Paired sample t-tests were run to compare the sleep variables assessed with the MCTQ between summer and winter. Wilcoxon Signed-rank tests were run to compare the BDI and PSQI scores between summer and winter.

Linear regression models were run to assess the influence of sex, age, chronotype (MSF_{sc}), season (summer vs. winter), weekly schedule (workdays vs. work-free days), and light exposure on DLMO, sleep timing, activity and on the phase relationship between DLMO and sleep. The average chronotype (between summer and winter) was used in these analyses. This choice was justified by a non-significant difference in chronotype between summer and winter (Table 1). Participant ID and season were analyzed as random factors to model the repeated measure design. Season was also analyzed as fixed factor together with the other predictors. Post hoc tests were done using Bonferroni correction for multiple comparisons.

The study was conducted according to the principles of the Medical Research Involving Human Subjects Act (WMO, 2012), and the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). The Medical Ethical Committee of the University Medical Center Groningen approved the study. The participants signed a written informed consent and received financial compensation for taking part in the study.

Results

The sleep timing characteristics of 33 participants (18 females; mean age 29 years \pm SD 4.9, age range: 23 - 40) assessed with the MCTQ in summer and in winter are reported in Table 1. In the same table, the BDI and PSQI scores are also reported. Only sleep onset on work-free days (MCTQ) was significantly different between seasons, being earlier (about 15 minutes) in winter compared to summer ($t(33) = -2.324$, $p = 0.0264$). The BDI scores did not significantly vary with season ($z = 1.050$, $p > .05$), while the PSQI scores were lower in winter compared to summer (mean winter: 3.7; mean summer: 4.4; $z = 2.044$, $p = 0.0406$). Lower scores in the PSQI correspond to better sleep quality.

Table 1. Sleep variables (assessed with the MCTQ) together with BDI and PSQI scores.

| Parameter | Summer (17.06.16-03.07.16) | | Winter (02.12.16-18.12.16) | |
|--------------------------------------|----------------------------|--------------|----------------------------|--------------|
| | Average (SD) | Range | Average (SD) | Range |
| Chronotype (MSF _{sc} ,h) | 4.24 (1.04) | 2.40 - 7.04 | 4.23 (1.01) | 2.04 - 6.24 |
| Social Jetlag (h) | 1.30 (0.76) | 0.23 - 3.13 | 1.38 (0.80) | 0.00 - 2.88 |
| Sleep onset on workdays (h) | -0.50 (0.60) | -1.92 - 0.75 | -0.67 (0.74) | -1.90 - 0.67 |
| Sleep end on workdays (h) | 6.99 (0.62) | 6.00 - 8.00 | 6.99 (0.69) | 5.50 - 9.00 |
| Sleep duration on workdays (h) | 7.49 (0.64) | 6.17 - 8.58 | 7.65 (0.69) | 6.20 - 8.83 |
| Sleep onset on work-free days (h) * | 0.47 (1.10) | -1.92 - 3.58 | 0.24 (0.87) | -1.42 - 2.33 |
| Sleep end on work-free days (h) | 8.67 (1.26) | 6.00 - 11.00 | 8.68 (1.38) | 5.50 - 10.50 |
| Sleep duration on work-free days (h) | 8.19 (1.02) | 4.92 - 9.83 | 8.44 (0.95) | 6.42 - 10.42 |
| BDI | 5.94 (5.33) | 0 - 22 | 5.15 (5.24) | 0 - 16 |
| PSQI * | 4.39 (2.21) | 1 - 9 | 3.67 (1.41) | 0 - 8 |

Data are reported separately for the summer and winter assessments. Data concerning chronotype, sleep onset and sleep end refer to external clock time and are reported in decimals (clock times before midnight are expressed with negative numbers). Significant differences between summer and winter are indicated with an asterisk (paired-sample t-test, $p < .05$). Sleep onset on work-free days was earlier in winter and subjective sleep quality (lower PSQI score) was better in winter.

Circadian phase assessment (DLMO)

We first ran a simple model with age, sex, chronotype (MSF_{sc}), season, and weekly schedule as predictors to explain the variance in DLMO. Age, chronotype, and weekly schedule were significantly associated with DLMO (age: $b = -0.088$, $t(27.04) = -2.993$, $p = 0.0059$; MSF_{sc} : $b = 0.416$, $t(27.57) = 3.005$, $p = 0.0056$; weekly schedule: $b = -0.424$, $t(57.13) = -4.567$, $p < .0001$). Older participants and earlier chronotypes had an earlier DLMO. In addition, DLMO was earlier on workdays compared to work-free days. DLMO did not vary with sex or season (sex: $b = 0.053$, $t(27.07) = 0.193$, $p > .05$; season: $b = -0.163$, $t(28.99) = -1.483$, $p > .05$).

We then ran the same model adding some interaction effects between the predictors. The interaction effects between season and weekly schedule and between season and chronotype were not significant (season*weekly schedule: $F_{1,55.575} = 0.258$, $p > .05$; season*chronotype: $F_{1,30.155} = 0.964$, $p > .05$). The interaction effect between chronotype and weekly schedule was significant ($F_{1,58.661} = 9.329$, $p = 0.0034$), meaning that the previously described main effect of weekly schedule on DLMO was modulated by chronotype. To explore this interaction effect, post hoc tests were done in three equal-sized chronotype groups (early: $MSF_{sc} < 3.8$, intermediate: $3.8 < MSF_{sc} < 4.6$; late: $MSF_{sc} > 4.6$). The difference in DLMO between workdays and work-free days was not significant in early and intermediate chronotypes (early: $b = 0.2$, $t(57) = 1.02$, $p > .05$; intermediate: $b = 0.3$, $t(54.8) = 2.33$, $p > .05$). In contrast, late chronotypes showed an earlier DLMO on workdays ($b = 0.8$, $t(56.5) = 5.11$, $p < .0001$), with the estimated difference in DLMO between workdays and work-free days being of almost 1 hour (Fig. 2).

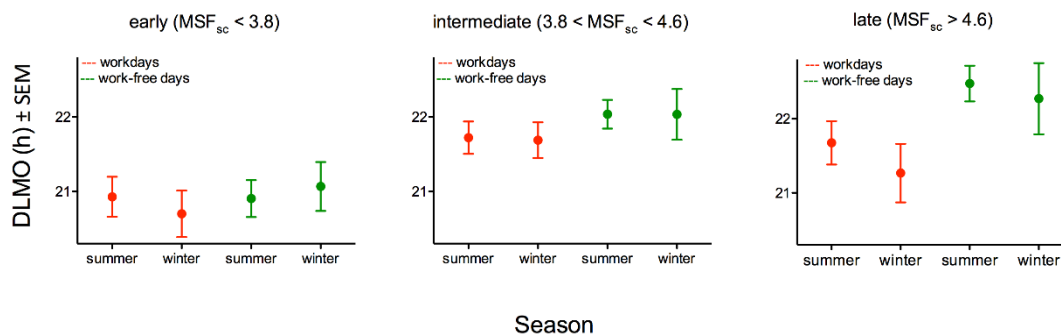


Figure 2. Chronotype-dependent variation in DLMO between workdays and work-free days.

Data points represent means with standard error of the mean (SEM) of DLMOs assessed on workdays (in red) and on work-free days (in green) in summer and in winter. Data are plotted separately for early ($MSF_{sc} < 3.8$), intermediate ($3.8 < MSF_{sc} < 4.6$), and late ($MSF_{sc} > 4.6$) chronotypes. DLMO was significantly earlier on workdays only in late chronotypes (independent of season).

To further explain the variation in DLMO, light exposure (assessed via actigraphy) was added to the model. Light exposure between 6:00 h and 12:00 h (morning light), between 18:00 h and 00:00 h (evening light), and during the all 24 hours (daily light) were first log transformed and then analyzed as separate predictors. First time of exposure to intensity levels above 100 lux was also added as predictor to the model. In this model, age, chronotype, and weekly

schedule were still significantly associated with DLMO. Among the light variables, only morning and the 24-hour light exposure were significantly related to DLMO (morning light: $b = -0.957$, $t(80.53) = -3.526$, $p = 0.0007$; daily light: $b = 0.896$, $t(82.2) = 2.756$, $p = 0.0072$). The model predicts an earlier DLMO with increased morning light exposure and a later DLMO with increased daily (24 hour) light exposure. To compare the strength of the influence of all the significant predictors on DLMO, we calculated the standard coefficients (β). Positive and negative β values indicate that an increase in the value of the predictor is associated with a later and earlier DLMO respectively. Morning and daily light exposure were the strongest predictors (morning light: $\beta = -0.491$; daily light: $\beta = 0.477$), followed by age ($\beta = -0.365$), chronotype ($\beta = 0.311$), and weekly schedule (workdays relative to work-free days: $\beta = -0.144$).

Sleep (assessed via sleep diaries)

As for DLMO, sleep (onset, end, and duration) did not significantly change between summer and winter. Sleep was earlier and shorter on workdays compared to work-free days (sleep onset: $b = -0.760$, $t(534.4) = -6.769$, $p < .0001$; sleep end: $b = -1.285$, $t(518.2) = -14.977$, $p < .0001$; sleep duration: $b = -0.512$, $t(520.7) = -4.871$, $p < .0001$). Further, morning light was associated with both sleep onset and sleep end, with increasing morning light exposure predicting earlier sleep timing (sleep onset: $b = -0.581$, $t(539.4) = -4.127$, $p < .0001$; sleep end: $b = -0.629$, $t(522.1) = -5.793$, $p < .0001$). Overall daily (24 hour) light exposure was also related to both sleep onset and sleep end, with increased light exposure predicting later sleep (sleep onset: $b = 0.378$, $t(541.1) = 2.067$, $p = 0.0392$; sleep end: $b = 0.510$, $t(535.2) = 3.576$, $p = 0.0004$). Evening light exposure was not significantly associated to sleep. The time of exposure to first morning light levels (above 100 lux) was associated with both sleep end and sleep duration (sleep end: $b = 0.156$, $t(539.6) = 4.688$, $p < .0001$; sleep duration: $b = 0.150$, $t(538.6) = 3.642$, $p = 0.0003$). Exposure to light levels above 100 lux later in the day was associated with later sleep end and longer sleep duration. Finally, males slept significantly later and shorter compared to females (sleep onset: $b = 0.419$, $t(23.9) = 2.691$, $p = 0.0128$; sleep duration: $b = -0.508$, $t(25.3) = -2.559$, $p = 0.0168$).

Phase relationship between DLMO and sleep (onset and end)

The phase angle difference between DLMO and sleep was calculated by subtracting sleep onset and sleep end from DLMO (negative values indicate that DLMO occurred earlier than sleep onset and sleep end, mean phase angle DLMO - sleep onset: -2:52 h; mean phase angle DLMO - sleep end: -10:22 h). Light exposure was not associated with the phase relationship between DLMO and sleep, and was therefore excluded from the model. The influence of sex, age, chronotype, weekly schedule and seasons were further analyzed. The phase relationship between DLMO and sleep onset varied with age, chronotype, and weekly schedule (age: $b = -0.095$, $t(26.09) = -3.315$, $p = 0.0027$; chronotype: $b = -0.313$, $t(27.05) = -2.289$, $p = 0.0301$; weekly schedule: $b = 0.632$, $t(83.89) = 4.221$, $p < .0001$). The phase angle difference was greater in older participants, in late chronotypes, and on work-free days compared to workdays. The model predicts that the phase angle difference between DLMO and sleep onset increased by 30 minutes on work-free days.

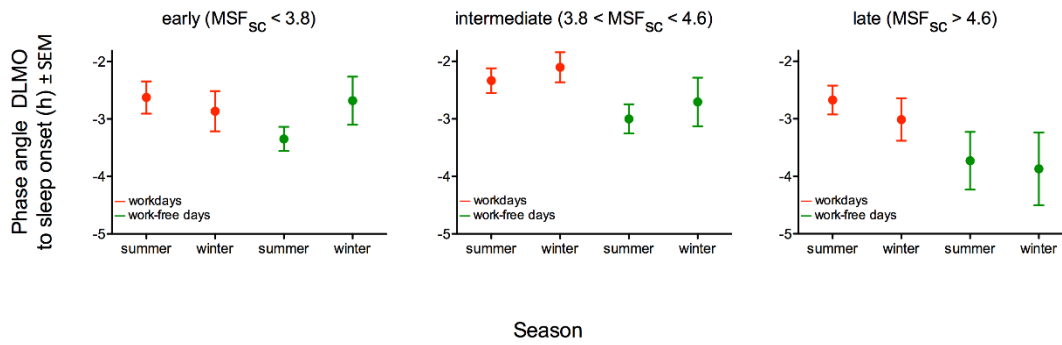


Figure 3. Chronotype-dependent variation in phase angle difference between DLMO and sleep onset on workdays and on work-free days.

Data points represent means with standard error of the mean (SEM). The phase angle difference between DLMO and sleep onset was calculated by subtracting sleep onset from DLMO. Data are plotted separately for early ($MSF_{sc} < 3.8$), intermediate ($3.8 < MSF_{sc} < 4.6$), and late ($MSF_{sc} > 4.6$) chronotypes. The phase angle difference between DLMO and sleep onset was greater on work-free days relative to workdays only in intermediate and late chronotypes (independent of season).

As for DLMO, adding the interaction effect between chronotype and weekly schedule to the model ($F_{1,82.168} = 6.516$, $p = 0.0125$) revealed that the weekly variation in phase angle difference between DLMO and sleep onset was modulated by chronotype. Post hoc tests showed that only intermediate and late chronotypes had a significant greater phase angle difference between DLMO and sleep onset on work-free days relative to workdays (early: $b = -0.3$, $t(56.8) = -1.10$, $p > .05$; intermediate: $b = -0.6$, $t(54.7) = -2.64$, $p = 0.033$; late: $b = -1.0$, $t(56.7) = -3.62$, $p < .0001$; Fig. 3). The model predicts that for late chronotypes the phase angle difference between DLMO and sleep onset was 1 hour greater on work-free days relative to workdays.

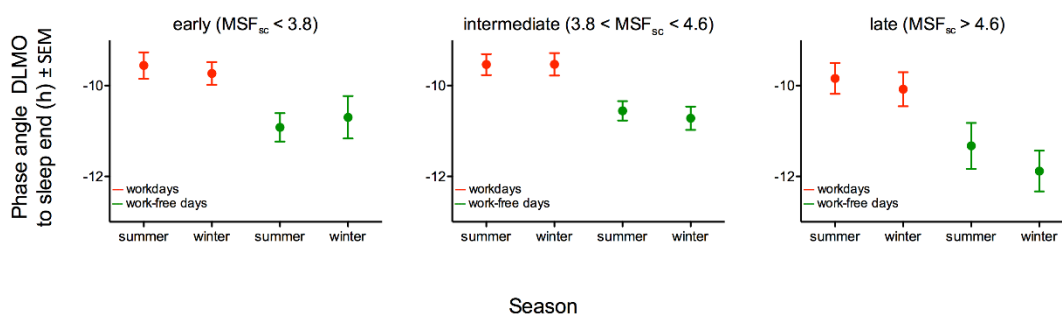


Figure 4. Chronotype-dependent variation in phase angle difference between DLMO and sleep end on workdays and on work-free days.

Data points represent means with standard error of the mean (SEM). The phase angle difference between DLMO and sleep end was calculated by subtracting sleep end from DLMO. Data are plotted separately for early ($MSF_{sc} < 3.8$), intermediate ($3.8 < MSF_{sc} < 4.6$), and late ($MSF_{sc} > 4.6$) chronotypes. The phase angle difference between DLMO and sleep end was greater on work-free days relative to workdays in all participants (independent of season), but this difference was more pronounced in late chronotypes.

Similarly, the phase relationship between DLMO and sleep end was greater in older participants ($b = -0.068$, $t(26.71) = -2.131$, $p = 0.0424$) and greater on work-free days ($b = 1.310$, $t(84.18) = 10.083$, $p < .0001$). The interaction effect between chronotype and weekly schedule was again significant ($F_{1,82.266} = 6.723$, $p = 0.0113$). Post hoc tests showed that all participants had a significant greater phase angle difference between DLMO and sleep end on work-free days relative to workdays (early: $b = -1.2$, $t(83.1) = -5.09$, $p < .0001$; intermediate: $b = -1.1$, $t(83.3) = -5.44$, $p < .0001$; late: $b = -1.8$, $t(83.9) = -7.31$, $p < .0001$; Fig. 4). However, the model predicts that for late chronotypes the phase angle difference between DLMO and sleep end was almost 2 hours greater on work-free days relative to workdays, whereas this difference was 1 hour for early chronotypes.

Center of gravity of activity

Center of gravity can be used as a phase marker of activity and indicates the time point when the amount of activity before and after is the same. Like with the other phase markers, season did not influence activity. Center of gravity of activity was later in late chronotypes and on work-free days (chronotype: $b = 0.512$, $t(34.4) = 4.254$, $p = 0.0002$; weekly schedule: $b = -0.360$, $t(521.3) = -2.463$, $p = 0.0141$; Fig. 5).

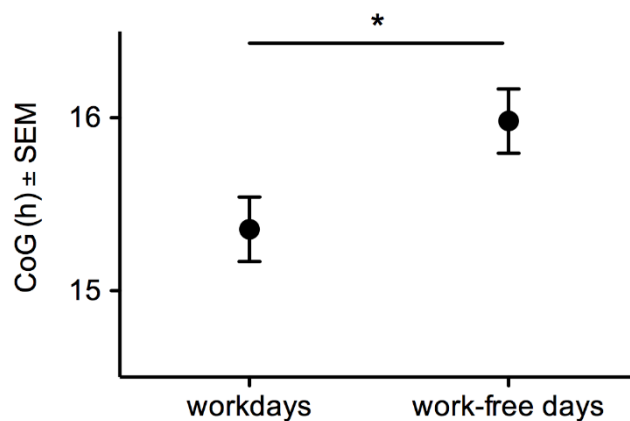


Figure 3. Center of gravity (CoG) of activity on workdays and on work-free days.

Data points represent means with standard error of the mean (SEM). CoG was earlier on workdays compared to work-free days (* $p < .05$).

Morning light and evening light were both associated with the phase of activity with opposite effects (morning light: $b = -0.548$, $t(524.3) = -2.973$, $p = 0.0031$; evening light: $b = 0.675$, $t(536.8) = 4.972$, $p < .0001$). The model predicts that increased morning light exposure advanced phase of activity and increased evening light exposure delayed it. The interaction effects between chronotype and season and between chronotype and weekly schedule were not significant (chronotype*season: $F_{1,32.19} = 0.208$, $p > .05$; chronotype*weekly schedule: $F_{1,510.84} = 1.524$, $p > .05$).

Light exposure

Figure 6 shows the light profile of the participants in summer and in winter and separately for workdays and work-free days. The effect of chronotype, weekly schedule, season and the interaction effects between chronotype and weekly schedule and chronotype and season were analyzed in two models: one aiming to explain the variation in morning light exposure and the other aiming to explain the variation in evening light exposure. We focused on the light exposure at these times of the day because that is when the circadian system is more sensitive to phase shifts.

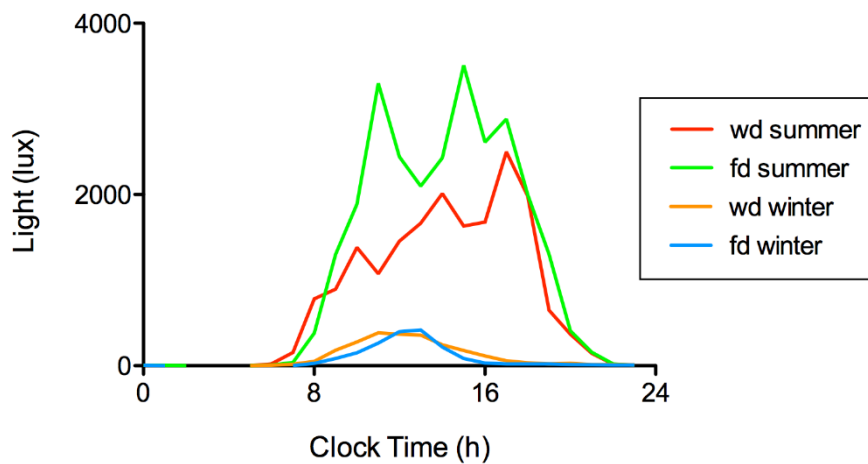


Figure 6. Light profile of all participants.

The average light intensities were calculated per hourly bins separately for workdays in summer (red), work-free days in summer (green), workdays in winter (orange), and work-free days in winter (blue). In summer participants were exposed to more light. On workdays participants were exposed earlier to light (probably because of commuting to work).

For morning light exposure, the interaction effects between chronotype and season and between chronotype and weekly schedule were significant (chronotype*season: $F_{1,31.19} = 13.400$, $p = .0009$; chronotype*weekly schedule: $F_{1,531.66} = 32.149$, $p < .0001$). To better interpret the significant main effects of season and weekly schedule on morning light exposure (season: $F_{1,31.08} = 41.267$, $p < .0001$; weekly schedule: $F_{1,531.76} = 15.370$, $p < .0001$), post hoc tests were done in three equal-sized chronotype groups (early: $MSF_{sc} < 3.8$, intermediate: $3.8 < MSF_{sc} < 4.6$; late: $MSF_{sc} > 4.6$).

All participants (trend for early chronotypes) were exposed to more morning light on workdays compared to work-free days (early: $b = -0.2$, $t(570) = -2.52$, $p = 0.06$; intermediate: $b = -0.4$, $t(575.3) = -5.15$, $p < .0001$; late: $b = -0.6$, $t(566.5) = -7.77$, $p < .0001$). However, the model indicates that this difference was greater for late chronotypes ($b = -0.6$). In addition, while there was no significant difference in morning light exposure on workdays between early and late chronotypes ($b = 0.1$, $t(39.2) = 1.08$, $p > .05$), late chronotypes were exposed to significantly less morning light on work-free days ($b = 0.6$, $t(49.9) = 4.53$, $p < .0001$).

All participants were exposed to more morning light in summer compared to winter (early: $b = 0.8$, $t(27.7) = 11.13$, $p < .0001$; intermediate: $b = 0.7$, $t(27) = 9.47$, $p < .0001$; late: $b = 0.4$, $t(27.9) = 4.40$, $p < .0001$). Early chronotypes were exposed to more morning light compared to late chronotypes, but this was significant only in summer and not in winter (summer: $b = 0.6$, $t(44.5) = 4.48$, $p < .0001$; winter: $b = 0.1$, $t(41.8) = 1.17$, $p > .05$). Figure 7 shows the light profiles for early ($MSF_{sc} < 3.8$) and late ($MSF_{sc} > 4.6$) chronotypes.

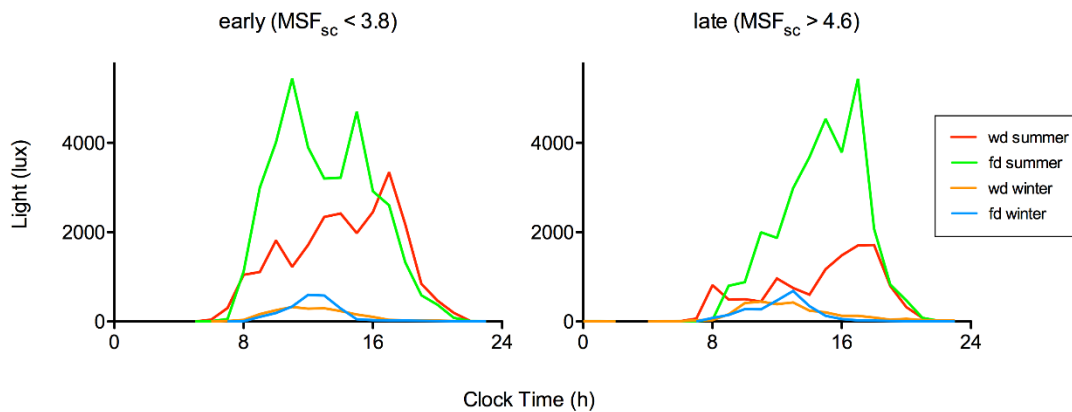


Figure 7. Light profile of early and late chronotypes.

The average light intensities were calculated per hourly bins separately for workdays in summer (red), work-free days in summer (green), workdays in winter (orange), and work-free days in winter (blue). The model indicates that late chronotypes were exposed to less morning light (between 6:00 h and 12:00 h) on work-free days compared to workdays. In addition they were exposed to light later in the day. This is particularly evident in summer where the peak in light exposure on work-free days is later compared to early chronotypes.

There were no significant interaction effects between chronotype and season nor chronotype and weekly schedule with respect to evening light exposure. The only significant change in evening light exposure was between seasons, with less evening light exposure in summer ($b = -1.660$, $t(56.5) = -5.612$, $p < .0001$).

Finally, we looked at when participants were first exposed to light levels higher than 100 lux. The interaction effects between chronotype and season and chronotype and weekly schedule were significant (chronotype*season: $F_{1,29.76} = 10.595$, $p = 0.0028$; chronotype*weekly schedule: $F_{1,518.24} = 5.189$, $p = 0.0231$). All participants were exposed to intensity levels higher than 100 lux earlier on workdays compared to work-free days (early: $b = 1.2$, $t(547) = 6.96$, $p < .0001$; intermediate: $b = 1.6$, $t(549.6) = 9.19$, $p < .0001$; late: $b = 1.9$, $t(548.2) = 8.77$, $p < .0001$). The model predicts that, while early chronotypes were exposed for the first time to light (>100 lux) only 1 hour later on work-free days compared to workdays, this difference was almost 2 hours for late chronotypes. When comparing early and late chronotypes there was no significant difference in the time of their first exposure to light on workdays ($b = -0.5$, $t(38.9) = -1.91$, $p > .05$), while late chronotypes were exposed significantly later to light on work-free days ($b = -1.2$, $t(56.3) = -4.00$, $p < .0001$). When looking at season, only early chronotypes and intermediate chronotypes were significantly

exposed to light later in winter compared to summer (early: $b = -1.5$, $t(26.8) = -7.52$, $p < .0001$; intermediate: $b = -1.3$, $t(25.6) = -6.33$, $p < .0001$; late: $b = -0.4$, $t(28.4) = -1.49$, $p > .05$). Similar to the morning light exposure, only in summer were early chronotypes exposed to light earlier in comparison to late chronotypes (summer: $b = -1.4$, $t(47.5) = -4.85$, $p < .0001$; winter: $b = -0.3$, $t(48.5) = -0.99$, $p > .05$).

Discussion

The aim of this study was to better understand entrainment in real life conditions by assessing several phase markers of the circadian clock and by looking at how the timing of light exposure impacts them. We assessed dim-light melatonin onset (DLMO), sleep, and activity at approximately the 21st of June (longest photoperiod: about 17 hours) and the 21st of December (shortest photoperiod: about 7 hours). In addition to season, we assessed the influence of weekly schedule on these markers, knowing that human behavior (e.g. sleep-wake cycle) can substantially differ between workdays and work-free days, leading to variations in light exposure and possibly in DLMO. Our expectation was that melatonin would be relatively robust to weekly changes but would reflect seasonal changes in photoperiod.

Influence of weekly schedule on circadian phase

The most striking results were obtained when looking at the influence of the weekly schedule on DLMO, sleep and activity. A later and longer sleep time on work-free days (especially in late chronotypes) has been described (Roenneberg et al., 2007a; Roenneberg & Mellow, 2007). This difference between the timing of sleep on workdays and on work-free days can be quantified by assessing social jetlag (Wittmann et al., 2006). Here, we also found a later and longer sleep as well as a later phase of activity on work-free days (relative to workdays), confirming previous observations about the weekly organization of the sleep-wake cycle in a school/working population (Wittmann et al., 2006). Additionally, we found that DLMO was later on work-free days compared to workdays and that this effect depended on chronotype. Early chronotypes ($MSF_{sc} < 3.8$) had similar DLMOs between workdays and work-free days, whereas late chronotypes ($MSF_{sc} > 4.6$) significantly delayed their DLMO (almost 1 hour) over the weekend. To the best of our knowledge this is the first observation of a chronotype-dependent delay in DLMO over the weekend in a working population where no restriction about sleep timing or light exposure was given. Similarly, the phase angle difference between DLMO and sleep onset varied according to weekday only in late chronotypes, being greater on work-free days.

In support of our findings, some experimental studies where sleep timing and/or duration were manipulated to simulate a typical weekend with later and longer sleep yielded the same results in terms of delay in DLMO (Burgess & Eastman, 2006; Crowley & Carskadon, 2010; Jelínková-Vondrasová et al., 1999; Taylor et al., 2008; Yang et al., 2001). The magnitude of the delay in these studies was similar to our observations, ranging from 30 minutes to 1 hour. In these studies, the authors suggested that a delay in DLMO following a change in the sleep schedule was probably related to differences in light exposure.

If someone sleeps later and wakes up later, he/she will be exposed to less morning light and to more evening light, which are both conditions that can induce a phase delay of the circadian clock. From the analysis of the light data, we could confirm this hypothesis and explain the dependency on chronotype of the weekend shift in DLMO based on different patterns of light exposure. Morning light exposure in early chronotypes did not differ significantly between workdays and work-free days (trend for more light on workdays). As expected, their DLMO did not significantly vary according to the day of the week. In contrast, late chronotypes were exposed to less and later morning light on work-free days, which apparently led to a later DLMO on the weekend. Similar differences in morning light exposure between chronotypes and between workdays and work-free days have been reported in other studies (Crowley et al., 2015; Goulet et al., 2007). Although evening (artificial) light is known to delay the clock and has received much more attention than morning light, our results suggest that morning light (between 6:00 h and 12:00 h) was more important in influencing phase of entrainment (assessed via DLMO). Morning light was in fact the strongest predictor of the variation in DLMO, while evening light (between 18:00 h and 00:00 h) was not significantly associated with DLMO. It is important to mention that the participants were exposed to overall more morning light than evening light (average morning light: 486 lux; average evening light 288 lux).

The influence of season on circadian phase

We did not find any clear seasonal variation in DLMO, sleep, or activity. This was somehow surprising, giving the remarkable difference in light exposure between summer (high intensity levels) and winter (low intensity levels) and the previously described influence of especially morning light on DLMO. Two recent studies did also not find any significant change in DLMO between summer and winter (Crowley et al., 2015; Stothard et al., 2017). When participants were assessed following one week in natural lighting conditions (camping), DLMO was still unaltered but the duration of secretion of melatonin was longer in winter compared to summer (Stothard et al., 2017). In electrical lighting conditions, this was not observed, suggesting that any seasonality in human physiology may be dampened by the modern life conditions. Although not significant, DLMO was earlier (about 15 minutes) in winter relative to summer but only on workdays. However, even if we had found a significantly earlier DLMO in winter, the finding could have derived from a longer secretion of melatonin in winter instead of an advance in phase of entrainment. For this reason, future seasonal studies should assess the full melatonin curve to also estimate peak melatonin as a possible better seasonal phase marker than DLMO.

It is important to mention that the summer assessments were done under daylight saving time (DST), while the winter assessments were done under standard zonetime (SZT) and no correction for the 1-hour delay in social time during DST was applied.

Conclusion

We here report a chronotype-dependent delay in DLMO on work-free days relative to workdays, with late chronotypes showing on average 1-hour delay in DLMO over the weekend. This variation in DLMO correlates with a different exposure to morning light,

which was significantly lower and later in late chronotypes on work-free days. In addition, it is possible that early and late chronotypes respond differently to light at different times of day, which could translate in more or less pronounced shifts in DLMO between workdays and work-free days. Individual differences in response to a light stimulus have been described, but whether there is a chronotype and/or a time-of-day dependency on sensitivity to light is not clear yet (Dijk et al., 2012; Santhi et al., 2011).

Despite the considerable changes in light exposure between summer and winter, we did not find any significant change in DLMO across seasons. This unexpected result could be explained by a gradual adaptation of the circadian system to high and low light intensities respectively in summer and winter. Laboratory studies have shown that the circadian clock adapts its sensitivity to low light intensities and can be phase shifted even with 0.5 lux (K. P. Wright et al., 2001). This change in sensitivity to light across seasons could help maintaining a stable phase of entrainment across seasons, which is an important feature of the clock since social schedules, for example, do not change with season.

These results could find application in everyday life. For instance, late chronotypes generally suffer from social jetlag, which has been previously associated with several health issues (Kantermann et al., 2013; Levandovski et al., 2011; Roenneberg, Allebrandt, Merrow, & Vetter, 2012; Wittmann et al., 2006; Wittmann, Paulus, & Roenneberg, 2010). However, effective interventions to decrease social jetlag have not been developed yet. Future studies could test the effects of increasing morning light specifically on work-free days to counteract the delaying effect of the weekend on DLMO. A study testing such a protocol in adolescents failed to find any counteracting effect of morning light on the delay in DLMO, but it would be interesting to repeat this study in adults (especially late chronotypes) since adolescents might have a reduced sensitivity to light (Crowley & Carskadon, 2010; Roenneberg et al., 2015).