Chronotype and environmental light exposure in a student population

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1 **<u>Abstract</u>**

2 In humans, and most other species, changes in the intensity and duration of light provide a

3 critical set of signals for the synchronisation of the circadian system to the astronomical day.

4 The timing of activity within the 24 h day defines an individual's chronotype i.e. morning,

5 intermediate or evening type. The aims of this study were to investigate the associations

6 between environmental light exposure, due to geographical location, on the chronotype of

7 university students. Over 6,000 university students from cities in the northern hemisphere

8 (Oxford, Munich and Groningen) and southern (Perth, Melbourne and Auckland) completed

9 the Munich ChronoType Questionnaire (MCTQ). In parallel, light measures (daily irradiance,

10 timing of sunrise and sunset) were compiled from satellite or ground stations at each of

11 these locations. Our data shows that later mid-sleep point on free days (corrected for

12 oversleep on weekends MFS_{sc}) is associated with: (i) residing further from the equator; (ii) a

13 later sunset; (iii) spending more time outside and (iv) waking from sleep significantly after

14 sunrise. However, and surprisingly, MSF_{sc} did not correlate with daily light intensity at the

15 different geographical locations. Although these findings appear to contradict earlier studies

16 suggesting that in the wider population increased light exposure is associated with an earlier

17 chronotype, our findings are derived exclusively from a student population aged between 17

18 and 26 years. We therefore suggest that the age and occupation of our population increase

19 the likelihood that these individuals will experience relatively little light exposure in the

20 morning whilst encountering more light exposure later in the day, when light has a delaying

21 effect upon the circadian system.

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<u>Introduction</u>

3   The circadian system adjusts physiology and behaviour to the varied demands of the day-
4   night cycle (Czeisler et al., 1999; Wright et al., 2001; Roenneberg et al., 2003). To ensure
5   synchrony with the astronomical day the circadian system entrains to daily environmental
6   signals (zeitgebers = time givers). The light-dark cycle is the most significant zeitgeber for
7   most organisms, including humans (Honma et al., 1987; Roenneberg et al., 2007).
8   Differences in the relationships between an individual's circadian phase and external local
9   time gives rise to a distribution of chronotypes across the population, ranging from *early*
10   chronotypes, the proverbial "larks", to *late* chronotypes termed "owls" (Roenneberg et al.,
11   2003).

12   The timing of light exposure has a differential effect upon circadian phase: early light
13   exposure advances the cycle whilst light late in the internal day delays circadian phase
14   (Czeisler et al., 1989; Khalsa et al., 2003). Thus exposure to bright artificial light in the
15   evening before bedtime has been associated with a delay in circadian phase as assessed by
16   measures of subjective chronotype (Martin et al., 2012; Vollmer et al., 2012); subjective
17   sleep timing (Koo et al., 2016); salivary melatonin levels (Gordijn et al., 1999; Benloucif et
18   al., 2008; Cajochen et al., 2011); and core body temperature (Krauchi et al., 1997).
19   Furthermore, adolescents living in urban areas and exposed to bright artificial light at night,
20   have a later chronotype as assessed by the Munich Chronotype Questionnaire (MCTQ) and
21   Morningness-Eveningness Questionnaire (MEQ), compared to those living in more rural
22   settings (Vollmer et al., 2012). By contrast exposure to bright light in the morning results in
23   an advance of the circadian phase of melatonin synthesis and release (Dijk et al., 1989;
24   Gordijn et al., 1999; Revell et al., 2005). In addition, bright morning light has been used as a
25   therapy for advancing sleep timings in patients with delayed sleep-wake phase disorders

1   (Rosenthal et al., 1990; Saxvig et al., 2014) and, more recently, with social jet lag (Geerdink

2   et al., 2016).

3   Despite society's increasing detachment from the natural light-dark cycle, sunlight can still

4   be seen to impact chronotype. Living further east within the same time zone in the Northern

5   hemisphere is associated with an earlier subjective chronotype in adults assessed using the

6   MCTQ (Roenneberg et al., 2007) and in adolescents assessed with the MEQ (Randler,

7   2008), most likely as a result of an earlier sunrise time. Seasonal changes are also apparent,

8   such that during the months of increasing day length, subjective chronotype advances with

9   individuals rising earlier (Kantermann et al., 2007; Allebrandt et al., 2014). There is also

10 some evidence that geographical location has an impact upon chronotype. For example, in a

11 study conducted in Brazil, subjective chronotype was assessed using the MCTQ and MEQ in

12 two cities: São Paulo at latitude 23° 32' S and longitude 46° 38' W and Natal at 05° 47' S

13 and 35° 12' W. Chronotype was found to be earlier in individuals living in Natal, the city

14 closest to the equator (Miguel et al., 2014).

15 Clearly the pattern of natural light within a particular environment will be critical in defining an

16 individual's phase of entrainment. However, an individual's behaviour within that

17 environment will also play an important role. A recent study compared the same individuals

18 living under their normal urban routines (including artificial light at night) with a period under

19 natural light exposure (camping without artificial light). The findings demonstrated that

20 increased exposure to natural light, advanced the circadian phase of all individuals (Wright

21 et al., 2013; Stothard et al., 2017). Increasing photic zeitgeber strength by spending more

22 time outside has also been correlated with self-reported chronotype: the more time spent

23 outside, the earlier the chronotype (Roenneberg&Merrow, 2007; Roenneberg et al., 2015).

24 By studying populations across the Northern and Southern hemisphere, specifically Oxford,

25 Groningen, Munich, Perth, Melbourne and Auckland, we aimed to investigate the association

26 between geographical location and chronotype and how different aspect(s) of environmental

1   light (timing; length of time spent outside; intensity of light, sleep timings relative to sunrise

2   and sunset) might influence chronotype.

3   **Materials and methods**

4   Students were recruited from six universities: University of Oxford, UK (51° 45′ N,

5   1° 15' W); University of Groningen, The Netherlands (53° 13′ N, 6° 33′ E); LMU, Munich,

6   Germany (48° 8′ N, 11° 34′ E); University of Western Australia, Perth, Australia (31° 57

7   ′ S, 115° 51′ E); Monash University, Melbourne, Australia (37° 48′ S, 144° 57′ E);

8   University of Auckland, New Zealand (36° 50′ S, 174° 44′ E). Students were asked to

9   complete the online version of the MCTQ twice, in May and October of 2010, to control for

10   seasonal influences. Overall 13,299 individuals completed the MCTQ online. Over half of the

11   participants were excluded from the analysis (see data processing). 6441 students (mean

12   age 21.5 ± 2.2 years, 67.5% female, see table 1 for group demographics) were included in

13   the analysis. Daily irradiance, sunrise and sunset times were obtained for May and October

14   2010. Ethical approval for this study was obtained from the local ethics committee for each

15   university involved in the study.

16   **Materials**

17   *The Munich Chronotype Questionnaire (MCTQ).* The online version of the MCTQ

18   (Roenneberg et al., 2003) was used in the native language of the country of each university.

19   The MCTQ consists of questions concerning sleep timings for both workdays and free days

20   separately, work time and time spent outside. The MCTQ has been validated against

21   actigraphic recordings (Vetter et al., 2015), and melatonin rhythms(Kitamura et al., 2014).

22   The MCTQ is used to calculate the MSF as the mid-point between sleep onset and sleep

23   end. MSF was corrected for oversleep on free days (MSF_{sc}: Mid Sleep point on Free days,

24   Sleep Corrected), that occurs as a result of sleep debt [MSF_{sc} = MSF – (SDf –

25   ((((nWD*SDw)+(7-nWD))*SDf)/7), where SDf is the sleep duration of free days, SDw is the

1   sleep duration of workdays and nWD is the number of workdays] (Roenneberg et al., 2004).

2   In cases where the numbers of workdays were missing, five workdays were assigned. Social

3   jet lag (SJL) was also calculated from the MCTQ [SJL=MSF-MSD] where MSF is the mid

4   sleep point of free days and MSD the mid sleep point of work days (Wittmann et al., 2006).

5   *Light data. 'Time spent outside'* was self-reported on the MCTQ. The weighted average of

6   the number of hours given for free days and workdays was calculated using the number of

7   workdays also reported on the MCTQ. If no workdays were given, 5 workdays were

8   assigned. [Time spent outside = ((time spent outside on workdays *number of workdays) +

9   (time spent outside on free days * (7-number of workdays)))/7].

10   *'Light dose'* is a measure of how much light individuals are exposed to over a given period of

11   time. Here, we calculated the average hourly irradiance for the day for participants that

12   completed the MCTQ and normalised this to the "time spent outdoors", averaged for work

13   and free days (Light dose = *daily irradiance / day length* * time spent outside].

14   *'Day length'* for each day of the collection periods, for each city, was calculated using the

15   world clock (http://www.timeanddate.com/worldclock).

16   *'Daily irradiances'* for the collection periods, for both May and October 2010, were obtained

17   from three sources on an hourly basis. The data for Oxford, Groningen and Munich were

18   provided by Dr. Lucien Wald (MINES, ParisTech), obtained from Meteosat satellite images

19   and converted to data maps of solar radiation using the Heliosat-2 method (Rigollier et al.,

20   2004). The data for Perth and Melbourne were obtained from the Australian Bureau of

21   Meteorology, again derived from satellite images processed by the Australian Bureau of

22   Meteorology. Finally, the data for Auckland were obtained from the New Zealand

23   Meteorological office based on readings from its ground station in Auckland. For all daily

24   irradiance, the data represent light intensity experienced at ground level, taking into account

25   weather conditions, either via processing of satellite data or as data taken at ground level.

26   **Data processing**

1   As only 440 participants completed the questionnaire in both May and October (420 from

2   northern hemisphere cities and 20 from southern hemisphere cities), longitudinal analysis

3   was not performed and data for these participants were only included in the data analysis

4   from the May collection period. Individuals were excluded if they were outside the age range

5   (17-26 years); did not indicate they were currently living in any of the cities of interest;

6   completed the questionnaire outside May or October 2010; or had reported working shifts

7   during the past three months. Individuals were also excluded if they indicated using an alarm

8   clock on free days (an exclusion criterion for chronotyping). For inter-hemispheric

9   comparisons, months were assigned to season (northern hemisphere, May and southern

10 hemisphere, October as spring and *vice versa* as autumn).

11 **Data analysis**

12 Statistical analysis was performed using R version 3.0.1 (2013-05-16, Copyright 2013 The R

13 Foundation for Statistical Computing). Linear mixed-effects models were fitted using the lme

14 package for group and seasonal assessments of MSF_{sc} and light data. For categorical

15 comparisons, linear models were referenced to Oxford for group comparisons, females for

16 sex comparisons and spring for season comparisons. Since age and sex are known to

17 influence chronotype, both of these were included as covariants when modelling MSF_{sc}.

18 Spearman's rank correlation analysis was performed to assess associations between MSF_{sc}

19 and light data.

20 **<u>Results</u>**

21 On average students in this sample reported the following habitual sleep-related times: bed

22 time on workdays: 00:11 ± 01:10 (mean, SD) and nearly an hour later on free days: 01:09 ±

23 01:24; wake-up time was two hours later on free days (09:45 ± 01:23) compared to

24 workdays (07:43 ± 01:06). A mean of 1.51 ± 0.93 hr of social jet lag was reported. Table 1

25 details wake-up and bed times for work and free days at each city along with social jet lag.

26 The sleep midpoint on free days (corrected for over sleep; MSF_{sc}) was different between

1   cities but not between seasons, and no city-season interactions were found (see suppl. data

2   model 1). Hence, MSF_{sc} was collapsed across seasons.

3   When plotting chronotype and time spent outside against the absolute distance of each city

4   from the equator, MSF_{sc} showed a positive association: with chronotype becoming later with

5   increasing distance (Fig. 1A). Controlling for age and sex, the cities formed three groups for

6   MSF_{sc}: Oxford, Groningen and Munich were not statistically different from each other, and

7   had the latest MSF_{sc}; Melbourne showed an intermediate MSF_{sc} and was statistically

8   significant from all the other cities; and Perth and Auckland showed the earliest MSF_{sc} and

9   were also not statistically significant from each other (see suppl. data model 2).

10   Overall, the students (regardless of city) reported they spend on average 2.20 (± 1.33) h

11   outside a day resulting in exposure to on average 24.74 (± 20.09) W/m^2 of light on the day

12   they completed the survey (light dose). Students in Perth reported spending the most

13   amount of time outside (2.76 ± 1.67 h) and experienced the highest intensity of light whilst

14   outside, light dose (62.68 ± 39.11 W/m^2, see suppl. data model 3 and 4). Whereas students

15   in Melbourne reported spending the least amount of time outside (1.89 ± 1.49 h), students in

16   Oxford received the lowest light dose (19.43 ± 14.31 W/m^2). In relation to geographical

17   location, the amount of time spent outside was not associated with the distance of each city

18   from the equator (Fig. 1B), but light dose did show an association, with the cities nearest to

19   the equator experiencing a higher light dose, except for Auckland (Fig. 1C).

20   MSF_{sc} was positively, although weakly, correlated with time spent outside (rho = 0.036, p =

21   0.005) indicating that the longer students spent outside the later their sleep midpoint on free

22   days. Time spent outside binned for MSF_{sc} in 30 min intervals, showed a stronger positive

23   correlation (rho = 0.86, p = 0.011, Fig. 2A). However, this was only statistically significant

24   with the removal of the outlier of MSF_{sc} binned from 06:30 to 07:00. Light dose was not

25   correlated with MSF_{sc} for raw (rho = -0.0005, p = 0.97) or binned data (rho = -0.15, p = 0.71,

26   Fig. 2B). Using a linear mixed effect model, taking age and sex into account, time spent

outside but not light dose was found to have a significant effect on MSF_{sc} (see suppl. data model 5). However, the addition of time spent outside into the model did not remove the effect of city, moreover no city-time spent outside interaction was found (see suppl. data model 6). This suggests that although the amount of time spent outside does have an influence on sleep midpoint on free days, other differences between the cities are also important.

To define the time of day the students in this population were most likely to receive natural light, the timing of sunrise and sunset the day each student completed the survey was compared to their self-reported sleep timings for work and free days. The proportion of daylight (i.e. between sunrise and sunset) during which time students were awake was negatively correlated to MSF_{sc} for both work (rho = -0.4, p < 0.001) and free days (rho = -0.71, p < 0.001, figure 2C), indicating that students with the latest MSF_{sc} were only likely to be awake for around 40% of the daylight period. This is because students wake up after sunrise rather than going to bed before sunset (Fig. 2D and 2E respectively). 75.5% of students woke up after sunrise on workdays and 98.1% on free days, with 15.7% waking up 5 hours after sunrise on free days (1.2% on workdays).

The impact of geographical location on MSF_{sc} was found to be most influenced by the timing of sunset. The average MSF_{sc} per city was plotted against the timing of sunrise and sunset for the day the survey was completed, as well as time spent outside and light dose (Fig. 3). The timing of sunset showed a positive association with MSF_{sc}: the later sunset the later MSF_{sc}. Interestingly no association was seen for sunrise. Collectively these data suggest that the timing of sunset and therefore the amount of light in the evenings may be more influential on sleep midpoint on free days than the amount of light in the morning in this population.

Discussion

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The findings from this study suggest that in a university student population a later chronotype is associated with: (i) living further from the equator; (ii) a later sunset; (iii) spending more time outside; and (iv) waking up after sunrise. Significantly, we did not find that light intensity was associated with chronotype. Initially, these findings appear to contradict earlier studies where increased light exposure is associated with an earlier chronotype in the general population (Roenneberg&Merrow, 2007; Wright et al., 2013; Roenneberg et al., 2015; Stothard et al., 2017). However, our findings are derived exclusively from a university student population aged between 17 and 26 years. Thus we suggest that the age and occupation of our population increase the likelihood that these individuals will experience relatively little light exposure in the morning whilst encountering more light exposure later in the day, when light has a delaying effect upon the circadian system.

In a sample of approximately 200,000 individuals from primarily Central Europe and North America, spending more time outside was associated with an earlier chronotype (Roenneberg&Merrow, 2007; Roenneberg et al., 2015). However, when age is taken into consideration, 15-20 year olds did not show a significant correlation between time spent outside and chronotype, and 20-25 year olds had only a weak correlation (Roenneberg et al., 2015). Our sample of over 6,000 students (17-26 years) falls across these age ranges, and also differs from the Roenneberg sample (MCTQ database) in several important aspects. A key difference is the work status of the populations studied: all individuals within our sample are university students during term time, the general population in the sample from the MCTQ database, would have included school students, university students and working individuals. It is possible, therefore, that imposed work schedules could result in more morning vs evening light exposure in the general MCTQ population. In addition, the data for the current study was collected exclusively in May and October, whilst the general population sample was collected all year round, which might also have an impact upon the timing of light exposure.

The timing of light exposure has a differential effect upon circadian phase: early light exposure advances the cycle whilst light late in the internal day delays circadian phase (Czeisler et al., 1989; Khalsa et al., 2003). In our student population we found that longer time spent outside the later chronotype, which would suggest that our population was exposed to more phase delaying evening light than phase advancing morning light. Although it was not possible to determine the timing of light exposure definitively from our study, we provide several lines of evidence that support the importance of evening light in this population. In the present study we demonstrated that the later students wake up after sunrise the later MSF_{sc}. As a result, individuals are likely to be exposed to a photoperiod with a greater proportion of evening phase delaying versus morning phase advancing light. Clearly, future studies will need to define the phase relationship between the internal circadian and external environmental light cycle. Moreover the timing of sunset, rather than sunrise was found to be most associated with MSF_{sc} in our population. Previously, a longitudinal study of around 55,000 individuals has reported that MSF_{sc} tracks sunrise and not sunset (Kantermann et al., 2007). However, again the broad demographics of this population make direct comparisons to our population difficult, but it is possible that the association of young adult university students (comparable to our population) is masked by other individuals in the sample. Finally the sensitivity of young adults to evening light has recently been demonstrated in two studies. In twenty healthy young adults (mean age 23) later light onset and offset has been associated with later melatonin onset as assessed using dim light melatonin onset (DLMO) (Wams et al., 2017). A mathematical model of sleep timings based on the experimentally derived effects of light on the human circadian clock and interaction of the circadian clock and sleep homeostat predicts a similar finding. Individuals with a longer intrinsic clock and hence later chronotype are predicted to be more susceptible to evening light, causing even more of a delay in the circadian cycle (Skeldon et al., 2017).

Geographical location was found to be associated with chronotype: the closer to the equator the earlier chronotype, and in this regard our findings are consistent with previous findings (Miguel et al., 2014). However, this association was assumed to be driven by higher environmental light intensities closer to the equator. Interestingly, it was only the duration of time spent outside - not the intensity of light - that was found to influence MSF_{sc} in our study except for subjects in Auckland. Although the intensity of light has been shown to impact on the entraining properties of light pulses under experimental conditions (Boivin et al., 1996; Zeitzer et al., 2005; Duffy&Czeisler, 2009), a saturation effect on shifting the phase of the melatonin rhythm has been reported above approximately 1000 lux (Zeitzer et al., 2000), equivalent to approximately 7.9 W/m^2 (based on the approximation that 1 lux = 0.0079 W/m^2 for solar irradiance). Considering the lowest average light intensity reported in this study was 19.43 W/m^2, and therefore well above saturation intensities, it is perhaps unsurprising that no effect of light intensity emerged. Instead in our population it appears that the association between chronotype and geographical location is due to the timing of sunset.

This study reported on a large sample of over 6,000 university students collected in term time during spring and autumn. Such numbers help mitigate the limitation of a cross-sectional assessment of chronotype. However, longitudinal studies are needed to determine precisely how an individual's chronotype changes with environmental light levels and age. Although based on self-reported sleep timings, the MCTQ is a validated measure of chronotype (Kantermann et al., 2015). The reliability of self-reported time spent outside as a proxy for light dose is less certain. The amount and type of environmental light exposure will be influenced by various factors including: photoperiod, weather conditions and the level of urbanisation. Although these have been taken into consideration in this study as much as possible (weather conditions accounted for in measures of daily irradiance and photoperiod in proportional assessment of daylight students awake for), objective assessment of time spent outside and light monitoring need to be undertaken to define when individuals go outside and the nature of their light exposure (inside vs outside). Furthermore, exposure to

artificial light was not addressed in this study, which of course will have an added impact on circadian physiology. Of particular interest in this young student population, is the impact of light-emitting devices on sleep and the circadian clock. Although, such devices have been found to impact sleep and circadian timing (Cajochen et al., 2011; Chang et al., 2015), the findings are mixed (Heath et al., 2014; Rangtell et al., 2016) and these changes are often small thus, the real world significance of these findings remains unclear (Zeitzer, 2015). The findings from this study however emphasise that environmental evening light exposure may need to be tailored for different populations. With the rapid growth in diversity of energy efficient light-emitting devices, robust, evidence-based advice is needed to ensure that individuals get the right kind of light at the right time of day to reinforce robust entrainment of the sleep-wake cycle.

In conclusion, we report that in this young adult university student population, time spent outside is associated with a later chronotype. This seems to be linked to the fact that this population spends more time outside in the evening, and that dusk light exposure will have a phase delaying effect upon their circadian biology. Moreover, we found that the closer students lived to the equator the earlier their chronotype. Significantly, this also appears to be associated with the timing of sunset rather than sunrise. Collectively our results emphasise the fact that the age and occupation of individuals will likely impact profoundly upon the timing of their light exposure and hence their phase of entrainment. Moreover, this work highlights the need for future longitudinal studies that will define these relationships with greater precision.

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