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

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From physiology to academic performance;
From students to working adults

Giulia Zerbini

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From physiology to academic performance;
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PhD thesis

to obtain the degree of PhD at the
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Table of contents

Chapter 1

General introduction

Giulia Zerbini

Clocks everywhere, but what time is it?

The rotation of the earth on its axis and around the sun determines regular changes in the environment, namely the alternation of day and night and of seasons. Many organisms have developed an internal time keeping mechanism in order to synchronize to external time signals (zeitgebers). The process that maintains a stable phase relationship between two oscillators is called entrainment (Aschoff, Klotter, & Wever, 1964). Having an internal clock able to entrain is thought to be adaptive since it allows, for example, anticipation of the regular changes in the environment (Moore-Ede, 1986). Light is considered the most important zeitgeber for human entrainment (Duffy & Wright, 2005; Roenneberg & Foster, 1997; Roenneberg, Kumar, & Merrow, 2007b; Skene, Lockley, Thapan, & Arendt, 1999; K. P. Wright et al., 2013). The internal clock has a period of about 24 hours (similar to the period of its zeitgeber) and is hence also called circadian clock (from Latin: circa diem = about a day).

In addition to light, there are several other zeitgebers that influence entrainment. For instance, food and physical activity have been shown to be able to entrain the behavior of animals even in the absence of light (Marchant & Mistlberger, 1996; Stephan, Swann, & Sisk, 1979). Non-photic entrainment has been described also in humans, although non-photic zeitgebers (e.g. physical activity, sleep-wake cycle, meal timing, social contacts) are much weaker time signals than is light (Mistlberger & Skene, 2005). Entrainment is therefore a complex phenomenon that can be challenged when the different time signals (external and internal) are not perfectly synchronized (Fig.1). For instance, different areas within a time zone have the same local clock time but different sun times (e.g. dawn in the eastern part of a time zone occurs earlier than in the western part of the same time zone). Similarly, daylight saving time shifts the social clock back and forth by 1 hour in spring and autumn, while sunset and sunrise times change gradually across the seasons. Shift-work is another example of how the social clock demands some individuals to be active at night when the circadian clock (in accordance to sun time) would promote sleep.

The main objectives of this thesis were to describe the negative consequences that can rise from conflicting internal and external time signals (part 1; chapters 2-5), to explore possible solutions to reduce the mismatch between the circadian and social clocks (part 2; chapter 6), and to better understand entrainment in real life conditions (part 3; chapters 7 and 8).

Variability in internal time

On top of the incongruences between different external time signals, internal time can vary substantially between individuals. Like many biological traits, also circadian clocks vary with individual characteristics such as sex, age, and genetic background (Hamet & Tremblay, 2006; Roenneberg, Kuehnle, Juda, Kantermann, et al., 2007a; Roenneberg et al., 2004). The additional exposure to different light landscapes results into a wide distribution of phases of entrainment, which determines, for instance, differences in sleep timing (Roenneberg & Merrow, 2007). These individual differences have been described as a distribution of chronotypes (Roenneberg, Kuehnle, Juda, Kantermann, et al., 2007a).

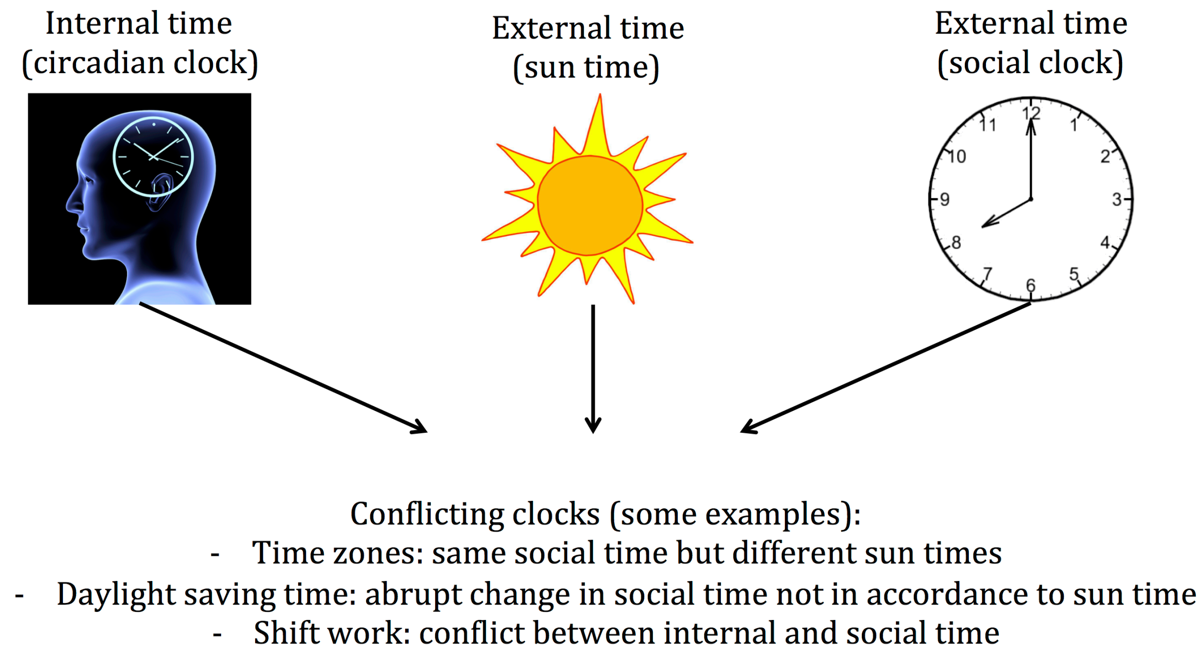

Figure 1. Internal time, sun time, and social time.
Internal and external time signals are not always perfectly synchronized in modern society, giving rise to several conflicts. Some examples of these conflicts are listed.

Chronotype and how to measure it

Chronotype is a feature of the circadian clock that can be easily measured via questionnaires such as the Munich ChronoType Questionnaire (MCTQ; Roenneberg, Wirz-Justice, & Merrow, 2003) and the Morningness-Eveningness Questionnaire (MEQ; Horne & Ostberg, 1976). Chronotype assessed via the MCTQ refers to sleep timing on work-free days, while the MEQ expresses chronotype as a diurnal preference towards morningness or eveningness. The answers to these questionnaires are highly correlated (r = -0.73) and show a variety of chronotypes ranging from very early (morning) to very late (evening) types (Zavada, Gordijn, & Beersma, 2005). In our studies, we use the MCTQ because expressing chronotype as a clock time gives more insight on the interaction between internal and external time.

With the MCTQ, chronotype is assessed as the midpoint of sleep on work-free days (MSF). For example, if one sleeps from 00:00 h to 08:00 h, MSF is 4. The majority of the working population (80%) needs alarm clocks to wake up on workdays (Roenneberg, Kantermann, Juda, Vetter, & Allebrandt, 2013); hence most people are chronically sleep deprived, showing sleep rebounds on work-free days to compensate for the lost sleep. Because of this tendency to oversleep on work-free days, MSF has to be corrected for the confounding influence of sleep debt accumulated on workdays, resulting in MSF sleep corrected (MSF_{sc}). This difference in sleep duration between workdays and work-free days is particularly evident in late chronotypes (if they have to attend early school/working schedules). Generally, the later the chronotype, the shorter the sleep duration on workdays and the longer the sleep duration on work-free days will be (Roenneberg, Kuehnle, Juda, Kantermann, et al., 2007a).

Characteristics of chronotype

Chronotype varies with age and sex. The prevalence of morning types is higher in the toddler age, but a progressive delay in chronotype is clear already during the first years of age (Randler, Faßl, & Kalb, 2017). Males on average are later than females, and this becomes particularly evident during adolescence (Randler et al., 2017; Roenneberg et al., 2004; Roenneberg, Kuehnle, Juda, Kantermann, et al., 2007a). Based on the MCTQ database, males reach their maximum in lateness at the age of 21, whereas females, who mature earlier, reach their maximum in lateness at the age of 19.5. After that age, both gradually become earlier chronotypes. When using another questionnaire to assess chronotype as diurnal preference (Composite Score of Morningness; Smith, Reilly, & Midkiff, 1989), these peaks in lateness are observed earlier (at the age of 18 for males and at the age of 15 for females; Randler et al., 2017).

Chronotype varies also with light exposure as shown by the correlation between chronotype and time of dawn described in a German population (Roenneberg, Kumar, & Merrow, 2007b). Moving from east to west, dawn was shown to progress continuously and the same was true for chronotype that was found to delay from east to west, although local clock time was the same within the given time zone. The correlation was stronger for smaller towns (less than 300,000 inhabitants), where people hypothetically experience a stronger zeitgeber since they spend more time outdoors and are exposed to more natural light than people living in bigger cities. This finding suggests the importance of considering sun time as well as total outside light exposure since the circadian clock seems to entrain to natural light rather than social schedules.

Genetic influences on chronotype have been also described in relation to extreme sleep behaviors, such as advanced and delayed sleep phase syndromes (Archer et al., 2003; Hamet & Tremblay, 2006).

Chronotype and other tools to assess phase of entrainment and sleep timing

Chronotype can be used to estimate an individual's phase of entrainment. Although chronotype is assessed with questionnaires (subjective measurement), the MCTQ asks about sleep timing that is usually reported quite objectively. The greatest advantage of using chronotype to assess phase of entrainment is the possibility to collect data in large populations in a quick and cost-effective way; the MCTQ online database has in fact reached over 200,000 entries so far.

Alternatively, biological (objective) phase markers can be used in human research to determine phase of entrainment, especially in relatively small-sample-size studies. Dim-light melatonin onset (DLMO) is often the first choice because melatonin has a robust and stable rhythm under the direct control of the circadian clock (Arendt, 2006; Klerman, Gershengorn, Duffy, & Kronauer, 2002). Melatonin is suppressed by light and therefore needs to be assessed in dim-light conditions. Other markers of the melatonin rhythm can be used, such as the peak in expression, but the advantage of DLMO is that it is accepted as a proxy for a full, overnight melatonin curve in most experiments (less expensive and time consuming). Importantly, chronotype, both assessed with the MCTQ and the MEQ, is generally strongly correlated with DLMO (MCTQ: $r = 0.68$; MEQ: $r = -0.70$; Kantermann, Sung, & Burgess, 2015).

Another biological phase marker mainly used in laboratory studies is core body temperature. Core body temperature also shows a strong circadian rhythm with a peak in the evening and a trough at night, but is more variable and influenced by external factors such as physical activity more than is melatonin (Klerman et al., 2002).

Sleep timing can be assessed both with daily sleep diaries (subjective measurement) and with actiwatches (objective measurement) that usually record activity together with light exposure. Actigraphy data can give also insights about sleep quality based, for instance, on awakenings and the time spent asleep in relation to time spent in bed (sleep efficiency). Actigraphy can also be used to assess other phase markers such as center of gravity (the time point when the amount of activity before and after is the same).

Conflicting clocks: consequences and possible solutions

Although individual differences in sleep timing and diurnal preferences have been widely described, society often imposes the same (early) social schedules on everyone, independent of their chronotype. This has consequences in terms of performance and health. For instance, a synchrony effect has been shown in literature, whereby early chronotypes perform better in the morning and late chronotypes perform better in the afternoon when tested with different cognitive tasks (Goldstein, Hahn, Hasher, Wiprzycka, & Zelazo, 2007; Lara, Madrid, & Correa, 2014; May, Hasher, & Stoltzfus, 1993). Similarly, there is a growing body of literature about the influence of chronotype on school performance. Students are expected to be at school early in the morning (some schools start at 7:00 h), while their circadian clock is considerably delaying during puberty (Crowley et al., 2014; Randler et al., 2017; Roenneberg et al., 2004). It is quite common for adolescents to have a chronotype around the same time when schools start, meaning that they are taught and take examinations in the middle of their biological night. This results in late chronotypes usually obtaining lower grades compared to early chronotypes (Borisenkov, Perminova, & Kosova, 2010; Escribano, Díaz-Morales, Delgado, & Collado, 2012; Randler & Frech, 2009; van der Vinne et al., 2015; Vollmer, Pötsch, & Randler, 2013). The interaction between chronotype and other factors important for school performance is complex and is further addressed in chapter 5, a review article about our and previous findings on this topic.

Social jetlag and health issues

The mismatch between the circadian and social clocks can be quantified by assessing social jetlag. The term social jetlag was coined by the group of Till Roenneberg in 2006 (Wittmann, Dinich, Merrow, & Roenneberg, 2006). Social jetlag is assessed with the MCTQ as the absolute difference between the midpoint of sleep on workdays (MSW) and on work-free days (MSF). MSW is a phase marker for sleep timing driven by the social clock, and MSF is a phase marker for sleep timing driven by the circadian clock. Therefore, the absolute difference between MSW and MSF is a measure of the discrepancy between the circadian and social clocks. Since social schedules start generally early in the morning, late chronotypes are the ones who suffer from social jetlag the most (Wittmann et al., 2006).

Social jetlag has been found to be associated with several health issues. Social jetlag significantly increases the probability of overweight and is positively associated with weight gain within this specific sub population (Roenneberg, Allebrandt, Merrow, & Vetter, 2012). Furthermore, stimulant consumption is related to social jetlag and, in particular, the greater the social jetlag, the more likely someone is a smoker (Wittmann et al., 2006). A positive correlation between social jetlag and depressive symptoms has also been found in a rural population in Brazil (Levandovski et al., 2011). Social jetlag is particularly high in shift workers and is positively correlated with heart rate, considered as a marker for cardiovascular diseases (Kantermann et al., 2013). Given all these findings, we hypothesized that a decrease in social jetlag could be beneficial in terms of improved health and performance, especially for those who experience a considerable discrepancy (more than 2 hours) between their circadian and social clocks. Finding practical and effective ways to decrease social jetlag was the second main objective of this thesis. Since social jetlag arises from a discrepancy between two clocks, there are two possibilities to decrease it: delay the social clock or advance the circadian clock. Several schools and working places have introduced delayed or flexible schedules, but still there are many situations in which late chronotypes need to perform at an early (non-optimal) time of day. Therefore, more studies investigating interventions to decrease social jetlag by modifying (advancing) phase of entrainment are needed.

How light influences the circadian clock and its entrainment

As previously described, light is the most important zeitgeber for human entrainment (Duffy & Wright, 2005; Roenneberg & Foster, 1997; Roenneberg, Kumar, & Merrow, 2007b; Skene et al., 1999; K. P. Wright et al., 2013). There are several characteristics of light that influence entrainment: wavelength, intensity, duration, time of day, and light history.

Almost two decades ago, a new opsin (melanopsin) was discovered in retinal ganglion cells (Provencio, Jiang, De Grip, Hayes, & Rollag, 1998). Melanopsin has a peak sensitivity around 470 nm (blue light) and is specifically responsible for the non-image forming effects of light, such as entrainment of the circadian clock (Brainard et al., 2001). Several studies have shown that blue light has the strongest effect on the circadian clock. For instance, melatonin suppression is higher after exposure to blue light compared to other colors (Brainard et al., 2015; Santhi et al., 2011; Thapan, Arendt, & Skene, 2001; H. R. Wright & Lack, 2009).

Other studies investigated the role of light intensity. Very low light intensities (1.5 lux) can entrain the human circadian clock in controlled laboratory conditions, but if the period of the light-dark cycle deviates from 24 hours (23.5 hours and 24.6 hours), higher light intensities are needed to achieve entrainment (K. P. Wright, Hughes, Kronauer, Dijk, & Czeisler, 2001). The response to light, in terms of melatonin phase shift and melatonin suppression, occurs in a dose-dependent manner. A single low light intensity pulse of 6.5 hours (below 15 lux for melatonin phase shift and below 80 lux for melatonin suppression) was found to trigger minimal responses in the circadian system (Zeitzer, Dijk, Kronauer, Brown, & Czeisler, 2000). With increasing light intensities, both phase shifting effects and melatonin suppression increased, reaching saturation above 200 lux for melatonin suppression and above 500 lux for melatonin phase shift (Zeitzer et al., 2000).

As for light duration, circadian phase shifts can be obtained with different light pulse durations. St Hilaire and colleagues (2012) showed that one hour of a bright white light pulse was sufficient to induce a phase shift of 2 hours, although it represented only 15% of a 6.7 hours light pulse, which, in a previous study, was shown to elicit a maximal phase shift (3 hours) of the circadian pacemaker (Khalsa, Jewett, Cajochen, & Czeisler, 2003; St Hilaire et al., 2012). Phase shifts of the circadian system have been also shown after exposure to a sequence of intermittent light pulses (Gronfier, Wright, Kronauer, Jewett, & Czeisler, 2004).

Time of day of light exposure is also an important factor. Light can have both advancing and delaying effects on the circadian clock. The phase response curve (PRC) describes the relationship between time at which a light pulse is presented and the direction of circadian phase shifts. The circadian system is more sensitive to light at the beginning and at the end of the biological night. In the first case, a light pulse induces phase delays, whereas in the second case the same light pulse induces phase advances (Khalsa et al., 2003).

Finally, the amount and intensity of light exposure (prior light history) during the day was shown to influence the sensitivity of the circadian system. For example, when the exposure to a light source followed a period in darkness or in dim light conditions, stronger responses in terms of phase shifts and melatonin suppression were found compared to when the same light pulse was applied after bright light exposure (Hebert, Martin, Lee, & Eastman, 2002).

Concept of decreasing social jetlag with light

Based on this literature, we developed two protocols involving light interventions to decrease social jetlag by modifying phase of entrainment and sleep timing.

The first protocol involved an increased exposure to (natural) morning light by sleeping with bedroom curtains open, and the second protocol involved a reduced exposure to (blue) evening light by wearing blue-light-blocking glasses. In both cases, we aimed to test the effectiveness of interventions that could be easily implemented in everyday life, since there is a lack of field studies confirming what has been already shown in controlled laboratory conditions.

We hypothesized that both the increased exposure to morning light and the reduced exposure to evening light would advance phase of entrainment and sleep timing, leading to longer sleep duration on workdays and therefore to a reduction of the sleep debt accumulated. This, in turn, would translate to less oversleep on work-free days, leading to a decrease in social jetlag via a better alignment of the midpoint of sleep on workdays and on work-free days (Fig. 2).

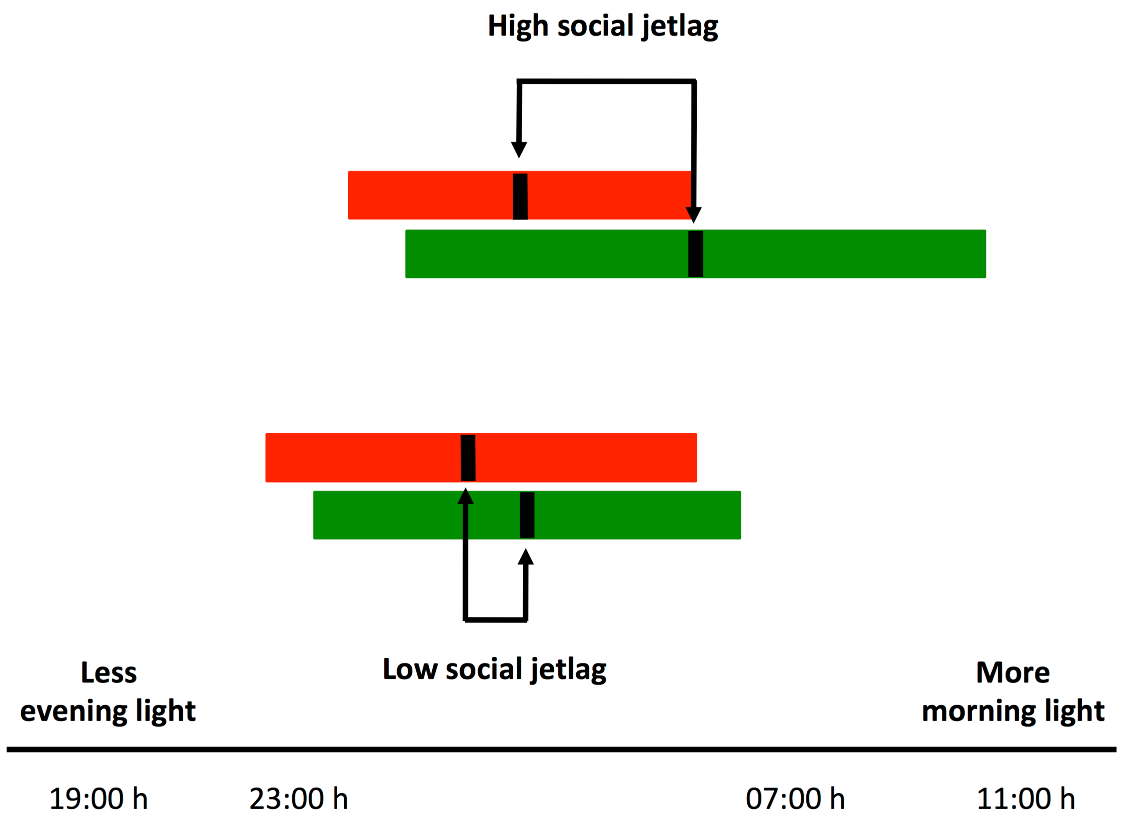

Figure 2. Decreasing social jetlag (SJL) with light.
The bars represent sleep duration on workdays (red) and on work-free days (green). The vertical black lines represent the midpoint of sleep on workdays (MSW) and on work-free days (MSF). SJL is the absolute difference between MSW and MSF. Light interventions involving less evening light and more morning light are both expected to advance sleep timing and phase of entrainment, leading to a longer sleep duration on workdays and therefore to a reduction of sleep debt accumulated. As a consequence, oversleep on work-free days is also expected to disappear. Altogether, this should result in a decrease of SJL via a better alignment of MSW and MSF.

Further understanding entrainment: the role of season and weekly schedule

Light is the primary zeitgeber for human behavioral entrainment, and therefore many studies have investigated the (isolated) effects of light on the circadian clock, often in highly controlled laboratory conditions. However, entrainment is a complex phenomenon resulting from the integration of many different internal and external time signals. Therefore, more field studies investigating entrainment in real life conditions may be useful to understand the problems and possibilities of giving sound advice to people who are not institutionalized.

At high latitudes, photoperiod (day length) varies substantially across seasons (e.g. in Amsterdam, The Netherlands (52° 22' N): summer photoperiod: 16:48 h and winter photoperiod: 7:40 h). This provides a unique opportunity to better understand entrainment in real life conditions by comparing, for instance, phase of entrainment between summer and winter. In summer, not only is photoperiod longer but also light intensity levels are generally higher. Increased light exposure was found to be associated to an earlier phase of entrainment, suggesting that phase of entrainment could be earlier in summer (Roenneberg & Merrow, 2007). Supporting this, sleep timing in humans was shown to track dawn by moving progressively to an earlier phase especially during the months of February and March when dawn comes minutes earlier each day (dawn on the 1st of February in Amsterdam: 8:21 h, dawn on the 31st of March 6:17 h) (Kantermann, Juda, Merrow, & Roenneberg, 2007).

It is important to note that in The Netherlands, like in many other countries in the world, daylight saving time (DST) is used during the summer months (April - October). During DST, social time is shifted one hour later. This was shown to disrupt entrainment and therefore might confound the findings from seasonal studies in humans (Kantermann et al., 2007).

The social clock also influences human behavior, in particular the sleep-wake cycle, but whether the social clock is able to change phase of entrainment is not clear yet. Sleep is usually later and longer on work-free days compared to workdays (social jetlag), and this difference is greater in later chronotypes (Wittmann et al., 2006). Because of the weekly schedule, workers are generally exposed to more morning light on workdays (Crowley, Molina, & Burgess, 2015). But is this difference in light exposure (only two work-free days a week) enough to phase shift the circadian clock every time over the weekend? It is possible that the sleep-wake cycle is quite flexible, but phase of entrainment remains stable.

Studies investigating the seasonal variation in the melatonin rhythm (as marker of phase of entrainment) have been inconclusive, probably because of the different conditions in which melatonin was assessed. Some have found no differences in DLMO, some an advance in melatonin peak in summer compared to winter, and some have found longer secretion of melatonin in winter compared to summer (Crowley et al., 2015; K. Honma, Honma, Kohsaka, & Fukuda, 1992; Illnerová, Zvolsky, & Vaněček, 1985; Stothard et al., 2017; Wehr, 1991).
Studies that have manipulated the sleep-wake cycle to simulate a typical weekend found a later DLMO associated with later and/or longer sleep (Burgess & Eastman, 2006; Crowley &

Carskadon, 2010; Jelínková-Vondrasová, Hájek, & Illnerová, 1999; Taylor, Wright, & Lack, 2008; Yang, Spielman, & Ambrosio, 2001). Therefore, the sleep-wake cycle seems able to feedback to the circadian clock and shift DLMO by probably changing the timing of light exposure between workdays and work-free days. However, whether this happens every week in a typical working population has not been shown yet.

Thesis overview

One of the main objectives of this thesis was to describe how conflicting internal and external clocks might result in negative consequences for human health and performance in order to suggest solutions. In particular, we focused on school performance in high-school students. We chose to study this population because chronotype delays during adolescence creating a conflict between the late circadian clocks of students and their early school schedules. In **chapters 2** and **3** we investigated the role of chronotype together with time of day (chapter 2) and school attendance (chapter 3) in determining school performance (grades). Previous literature had shown that late chronotypes obtain, on average, lower grades compared to early chronotypes. We expanded on this showing that the chronotype-effect on grades is complex, requiring a comprehensive assessment of the influence of chronotype together with other factors important for school performance, such as time of day and school attendance. In **chapter 4** we aimed to expand our previous results about the interaction effect between chronotype and time of day on grades. We chose university students as an interesting population because they are examined early in the morning as well as late in the evening. **Chapter 5** reviews the literature about chronotype and school performance with the aim of suggesting possible mechanisms behind a lower school performance in late chronotypes. Solutions to increase school performance in late chronotypes are also explored.

The second main objective of this thesis was to test the effectiveness of light interventions to decrease the mismatch between the circadian and social clocks (social jetlag). Light interventions were chosen for this purpose because light is the main zeitgeber for human entrainment and, if timed properly, it is capable of shifting (advancing) the circadian clock.
In **chapter 6** the findings from two studies are described. The light interventions implemented in these studies involved an increase in (natural) morning light exposure (by sleeping with bedroom curtains open) and a decrease in (blue) light evening exposure (by wearing blue-light-blocking glasses).

The final objective of this thesis was to better understand entrainment in real life conditions. We took advantage of the natural changes in photoperiod across seasons to assess how the variation in intensity and duration of light exposure might influence human behavior and entrainment. **Chapter 7** describes how school attendance and performance vary across seasons. Data were collected for two consecutive academic years. The role of photoperiod (day length) and of weather conditions was investigated in relation to the annual rhythm observed in school attendance. In **chapter 8** we investigated the influence of season (summer vs. winter) and weekly schedule (workdays vs. work-free days) on sleep timing, on phase of entrainment (DLMO), and on the relationship between these two parameters. The possible role of chronotype in influencing these variables was also investigated.

Finally, **chapter 9** summarizes the main findings of this thesis: the influence of chronotype on school performance and the effects of different light interventions and season on social jetlag, sleep timing, and phase of entrainment. The chapter integrates and connects these findings. The discussion focuses on late chronotypes, describing the challenges offered by early social schedules, the consequences in terms of impaired performance, and the possible solutions to decrease the mismatch between the circadian and the social clocks. In Figure 3 a schematic overview of this thesis is represented.

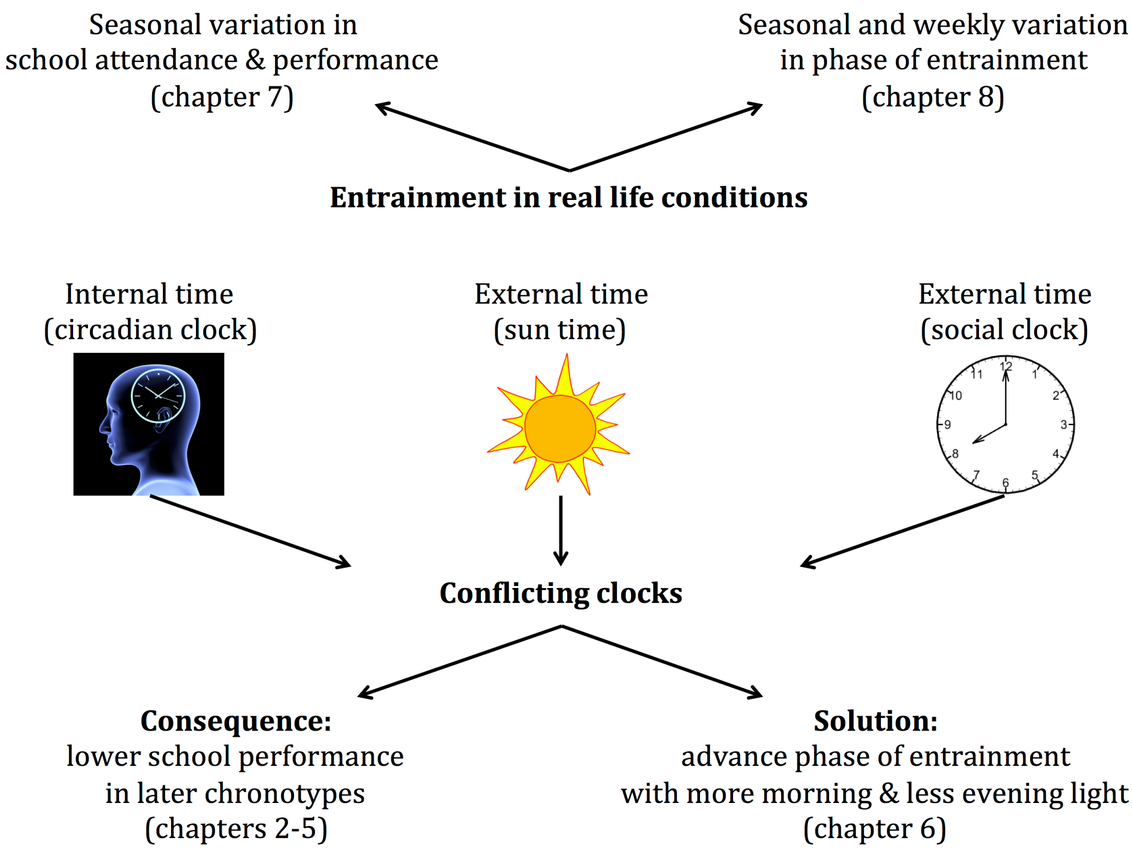

Figure 3. Schematic thesis overview.

Chapter 2

Timing of examinations affects school performance differently in early and late chronotypes

Vincent van der Vinne*, Giulia Zerbini*, Anne Siersema, Amy Pieper, Martha Merrow, Roelof A. Hut, Till Roenneberg, and Thomas Kantermann

* These authors contributed equally to this work.

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Abstract

Circadian clocks of adolescents typically run late – including sleep times – yet adolescents generally are expected at school early in the morning. Due to this mismatch between internal (circadian) and external (social) times, adolescents suffer from chronic sleep deficiency, which, in turn, affects academic performance negatively. This constellation affects students' future career prospects. Our study correlates chronotype and examination performance. In total, 4,734 grades were collected from 741 Dutch high school students (ages 11-18 years) who had completed the Munich ChronoType Questionnaire (MCTQ) to estimate their internal time. Overall, the lowest grades were obtained by students who were very late chronotypes (MSF_{sc} > 5.31 h) or slept very short on schooldays (SD_w < 7.03 h). The effect of chronotype on examination performance depended on the time of day that examinations were taken. Opposed to late types, early chronotypes obtained significantly higher grades during the early (08:15-09:45 h) and late (10:00-12:15 h) morning. This group difference in grades disappeared in the early afternoon (12:45-15:00 h). Late types also obtained lower grades than early types when tested at the same internal time (hours after MSF_{sc}), which may reflect general attention and learning disadvantages of late chronotypes during the early morning. Our results support delaying high school starting times as well as scheduling exams in the early afternoon to avoid discrimination of late chronotypes, and to give all high school students equal academic opportunities.

Introduction

School achievements determine academic opportunities and can have life-long consequences, for example, in terms of salaries (Baum, Payea, & Ma, 2013; French, Homer, Popovici, & Robins, 2015; Geiser & Santelices, 2007). Both sleep timing and duration are important factors influencing school performance (Curcio, Ferrara, & De Gennaro, 2006; Diekelmann & Born, 2010). According to the two-process-model, sleep is regulated by the interaction between a homeostat and the circadian clock (Borbély, 1982; Daan, Beersma, & Borbély, 1984). The homeostat refers to sleep pressure accumulating during wakefulness and decaying during sleep. While the circadian clock promotes wakefulness during the biological day, especially in its second half, it promotes sleepiness primarily in the second half of the biological night. Our chances to fall asleep are optimal when sleep pressure is high and the circadian clock decreases its wake promotion. In turn, we wake up most easily when sleep pressure has dissipated, and when the circadian clock ceases to promote sleep.

Like most biological traits, sleep timing varies between individuals. This variance is thought to reflect differences in how individual circadian clocks synchronize (entrain) to the light-dark cycle (Roenneberg & Merrow, 2007). Environmental signals to which circadian clocks entrain are called zeitgebers (Aschoff, Klotter, & Wever, 1964). Light is the most important zeitgeber for humans (Roenneberg, Kumar, & Merrow, 2007b; Wever, 1979), who vary in how early or late their circadian rhythms establish a stable 'phase of entrainment' in reference to the light-dark cycle (e.g., to dawn), resulting in different 'chronotypes' (Roenneberg, Kumar, & Merrow, 2007b). Besides being modified by light exposure, chronotype depends on genetic background and development (Roenneberg, Kuehnle, Juda, Kantermann, et al., 2007a).
The Munich ChronoType Questionnaire (Roenneberg, Wirz-Justice, & Merrow, 2003) assesses chronotype using simple, short questions about sleep timing on both workdays and work-free days. Chronotype is calculated from the midpoint of sleep on work-free days (MSF), corrected for sleep debt accumulated on workdays (MSF_{sc}). Chronotype can be used to estimate an individual's internal time in reference to external (social) time (Kantermann et al., 2012a; Vetter, Juda, & Roenneberg, 2012).

Chronotype of adolescents is typically later than in all other age groups, resulting in later sleeping times (Roenneberg, Kuehnle, Juda, Kantermann, et al., 2007a). Thus, early school starting times lead to chronic sleep deficiency in high school students (Carskadon, Wolfson, Acebo, Tzischinsky, & Seifer, 1998; Gibson et al., 2006; R. E. Roberts, Roberts, & Duong, 2009), a phenomenon that is associated with lower performance (Lo et al., 2012; Meijer, 2008; Perez-Lloret et al., 2013; Philip et al., 2012; Wolfson & Carskadon, 2003). The condition of chronic sleep deficiency associated with early work or school hours and late sleep onset has been called social jetlag (SJL; Wittmann, Dinich, Merrow, & Roenneberg, 2006). SJL quantifies the mismatch between internal and external time and correlates positively with chronotype (Wittmann et al., 2006). Increased SJL has been associated with lower academic achievement (Genzel et al., 2013; Haraszti, Ella, Gyöngyösi, Roenneberg, & Káldi, 2014), and late chronotypes obtain lower grades than early types (Borisenkov, Perminova, & Kosova, 2010). The same correlation is found when diurnal preferences are assessed by the Morningness-Eveningness Questionnaire (MEQ; Horne & Ostberg, 1976): again, evening types achieve lower grades than morning types (Beşoluk, Önder, & Deveci, 2011; Escribano, Díaz-Morales, Delgado, & Collado, 2012; Preckel et al., 2013; Randler & Frech, 2006).

The time of day at which examinations are taken could also influence examination outcomes because cognitive functions, including attention, fluctuate during the day (Escribano & Díaz-Morales, 2014; Haraszti et al., 2014; Higuchi, Liu, Yuasa, Maeda, & Motohashi, 2000; Knight & Mather, 2013). When different chronotypes are tested at the same external time, they are actually tested at different internal times. We therefore predict a chronotype-dependent time-of-day effect on grades. Here, we collected 4,734 grades from Dutch high school examinations performed between 08:15 and 15:00 h and assessed how school performance depends on external and internal time. To our knowledge, this is the first detailed description of chronotype-dependent fluctuations in grades across a typical school day.

Methods

This study was performed at a local high school in Coevorden, the Netherlands (52° 40' N, 6° 45' E). Our study was done 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). Research that does not subject people to procedures or does not require people to follow rules of behaviour is an exemption to this WMO act. In addition, retrospective research/patient file research (as our collection of grades here) does not fall under the WMO act. Based on the Dutch national regulations, our study was not invasive of participants' integrity, as it was performed during regular school hours. We also obtained written consent from the school principal confirming that our study was performed according to the principles of the Declaration of Helsinki. School grades from randomly distributed examinations in 16 subjects (art, biology, chemistry, Dutch, economics, English, French, geography, German, Greek, history, Latin, management, math, physics, sociology) were collected between September and November 2013. Grades were collected together with the time of day that each examination was taken during eight 45-minute lessons scheduled between 08:15 and 15:00 h or during examination weeks with modified schedules. Time-of-day dependent examination performance was assessed by comparing grades for all eight regular lessons.

Data collection performed in this study was done by simultaneously collecting 2 databases: one of examination grades and another with MCTQs. In the first half of October 2013, 741 students (364 male and 377 female; mean age 14.1 ± 1.7 SD; age range 11-18 years) filled in the MCTQ (Roenneberg et al., 2003). Of these, 700 were associated with at least 1 examination grade in the database, reflecting a large overlap of our 2 databases. The MCTQ provided information about sleep timing on work/schooldays and work-free days, as well as demographic information (age and sex). Each student's chronotype (MSF_{sc}), SJL (absolute difference between mid-sleep on work-free days and on work/schooldays) and sleep duration on work/schooldays (SD_w) was determined from the subjective entries to the MCTQ (Roenneberg et al., 2003). Because MSF_{sc}, SJL, and SD_w showed nonlinearity, categorical analyses were applied ranking all students for each of these 3 variables separately and divided these into 5 equal-sized groups. Additionally, regression analyses were performed for all three variables to ensure that significant differences observed in the categorical analyses did not the result from the subgroup selection.

The interaction between time of day and chronotype on grades was investigated by subdividing the population into 2 groups of early ($MSF_{sc} < 4$) and late ($MSF_{sc} > 4$) chronotypes. This cutoff was estimated in a preliminary analysis as the optimal critical MSF_{sc} of a 2-line regression fit using a constant grade for MSF_{sc} < critical MSF_{sc} and a constant slope for MSF_{sc} > critical MSF_{sc}. For the 2 groups, we compared grades obtained in the early morning (08:15-09:45 h), late morning (10:00-12:15 h), and early afternoon (12:45-15:00 h). We note that the first time slot (90 minutes) differs in length from the other 2 time slots (135 minutes each), which was necessary so that breaks fall between and not within these time periods. To assess the effect of internal time on performance, local examination times were converted to 'hours since MSF_{sc}'.

The Dutch grading system ranges from 1 (lowest) to 10 (highest). Grades of 5.5 or higher are needed to pass an examination. Grades in the current study were clustered around an average of 6.5 (>5.5: 12.2%; 5.5-6.5: 38.5%; 6.5-7.5: 34.3%; >7.5: 15%; (International Recognition Department of Nuffic Netherlands Organisation for International Cooperation in Higher Education, 2013).

Restricted maximum likelihood (REML) fitted mixed models with 'individual' (student ID), 'subject' and 'school year' as random factors were used in all analyses. These factors had a significant influence on grade while 'sex' and 'age' were excluded as co-factors since their effects did not reach significance. Age and school year were strongly correlated with grades. Because Dutch school grades tend to decline by school year as a reflection of increasing performance standards, school year was included in the statistical model. All statistical analyses were performed using SAS JMP 7.0 software. Tukey HSD post hoc tests were applied to perform pairwise comparisons for categorical variables. Error bars in all figures represent standard error of the mean derived from the statistical model.

Results

The demographics of our study population and the number of examinations collected in each of the eight lessons are shown in Table 1. The average number of grades collected per student was 7.1 ± 5.9 SD (range 1-23).

On the whole, later chronotypes obtained significantly lower grades compared to earlier types (544 students; 4,492 grades; $F_{4,520.6} = 3.864$; $p = 0.0042$; Fig. 1A). The average grades obtained by the 5 SJL subgroups used in our analysis were not significantly different (544 students; 4,492 grades; $F_{4,520.4} = 2.299$; $p = 0.0578$; Fig. 1B). Short sleep on workdays (SD_w) was also significantly associated with lower grades (580 students: 4,719 grades; $F_{4,546.6} = 4.615$; $p = 0.0011$; Fig. 1C). When analyzed as continuous variables instead of categorizing into 5 groups, MSF_{sc} (544 students; 4,492 grades; $F_{1,601.1} = 11.25$; $p = 0.0008$), SJL (544 students; 4,492 grades; $F_{1,586.1} = 8.585$; $p = 0.0035$) and SD_w (580 students; 4,719 grades; $F_{1,586.6} = 9.212$; $p = 0.0025$) were each significantly associated with grades.

Table 1. Demographics of 364 male and 377 female high school students and number of grades collected in each of the eight school hours.

Outcome measure	Average (± SD)	Range	Count
Age (years)	14.1 (1.7)	11 - 18	---
Chronotype (MSF_{sc})	4.44 (1.15)	-0.38 - 10.58	---
Social jetlag (h)	2.31 (1.01)	0.00 -7.21	---
Sleep duration school/workdays (SD_w)	7.85 (1.02)	3.25 - 10.67	---
Grades collected per student	7.1 (5.9)	1-23	---
Grades during regular lessons	---	---	3825
Grades during exam period	---	---	909
Grades in school hour 1 (0815 – 0900 h)	---	---	425
Grades in school hour 2 (0900 – 0945 h)	---	---	427
Grades in school hour 3 (1000 – 1045 h)	---	---	511
Grades in school hour 4 (1045 – 1130 h)	---	---	687
Grades in school hour 5 (1130 – 1215 h)	---	---	618
Grades in school hour 6 (1245 – 1330 h)	---	---	471
Grades in school hour 7 (1330 – 1415 h)	---	---	370
Grades in school hour 8 (1415 – 1500 h)	---	---	295

Time-of-day effects on school performance were assessed for all grades obtained for examinations during regular lessons (excluding grades from examination weeks with modified schedules). Average grades varied significantly with school hour (525 students; 3,804 grades; $F_{7,2773} = 6.150$; $p < 0.0001$; Fig. 2A). Grades from examinations taken during the 1st and 8th (last) school hour were significantly lower compared to grades from examinations taken during the 2nd and 7th school hour. Grades obtained in the early morning (08:15-09:45 h), late morning (10:00-12:15 h) and early afternoon (12:45-15:00 h) were assessed to investigate the overall influence of time of day on grades. Without taking chronotype into account, examination times did not affect school grades (525 students; 3,804 grades; $F_{2,2108} = 0.194$; $p = 0.8239$), but a time-of-day effect was significant when comparing early and late types (494 students; 3,639 grades; $F_{2,3551} = 4.171$; $p = 0.0155$; Fig. 2B).

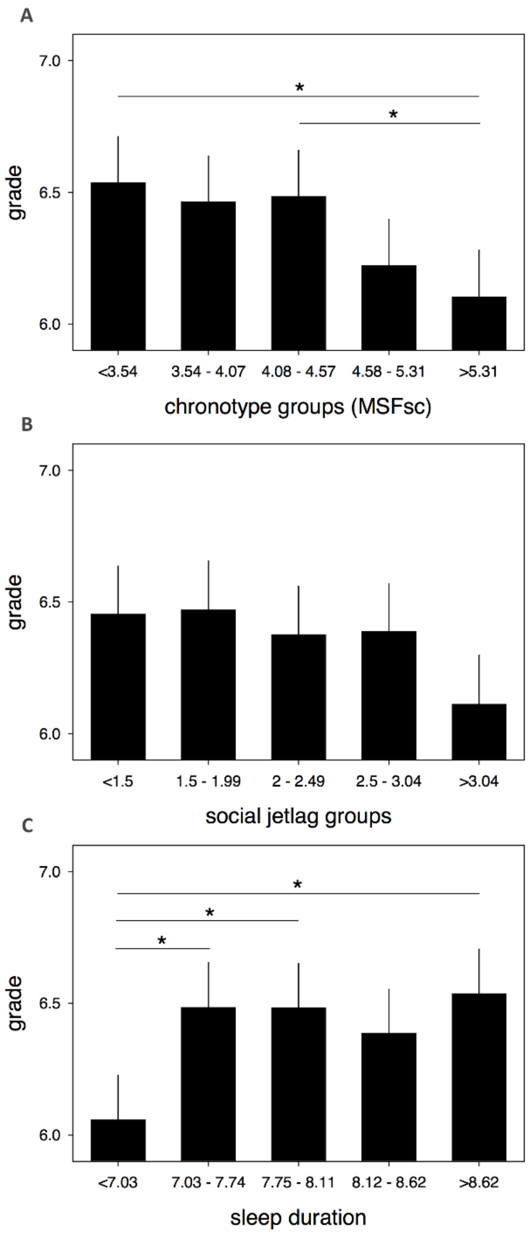

Figure 1. Effects of chronotype (MSF_{sc}), social jetlag (SJL) and sleep duration (SD_w) on grades. MSF_{sc}, SJL and SD_w are each grouped in 5 equal-sized groups (cutoffs provided under each bar). (A) Chronotype affected grades significantly. The latest 20% chronotypes obtained significantly lower grades compared with the earliest and middle 20%. (B) Social jetlag did not significantly affect grades. (C) SD_w significantly affected grades. Students sleeping shorter than 7.03 hours on schooldays obtained significantly lower grades compared with students sleeping longer. Examination grades vary between 1 (lowest) and 10 (highest) with 70% of grades between 5.5 and 7.5; >5.5 represents a passing grade. *$p < 0.05$

Early types obtained significantly higher grades during the early (08:15-09:45 h) and late (10:00-12:15 h) morning, but this difference disappeared in the early afternoon (12:45-15:00 h). The average difference in grades between early and late chronotypes disappeared in the early afternoon (early morning: 0.39; late morning: 0.26; early afternoon: 0.001), indicating that early and late types obtained similar grades in the early afternoon. Analysis of time-of-day as a continuous variable supported these findings (chronotype: 525 students; 3,804 grades; $F_{1,1283} = 0.219$; $p = 0.6397$; chronotype x time-of-day: 494 students; 3,639 grades; $F_{1,3559} = 7.676$; $p = 0.0056$).

Because chronotype varied in our population, examinations were taken at different internal times (*i.e.*, local examination times converted to hours after MSF_{sc}). Late types were examined at significantly earlier internal times compared to early types (early group: 8.6h; late group: 7.0 h; $F_{1,346} = 344.1$; $p < 0.0001$). The correlations between grades and internal time differed significantly between early and late types (494 students; 3,639 grades; $F_{1,3627} = 9.656$; $p = 0.0019$; Fig. 3) and revealed a negative slope for early (205 students; 1,704 grades; $F_{1,468.1} = 4.386$; $p = 0.0368$; slope = $-0.049\ h^{-1}$) and a positive slope for late types (289 students; 1,935 grades; $F_{1,895.3} = 6.746$; $p = 0.0095$; slope = $0.055\ h^{-1}$).

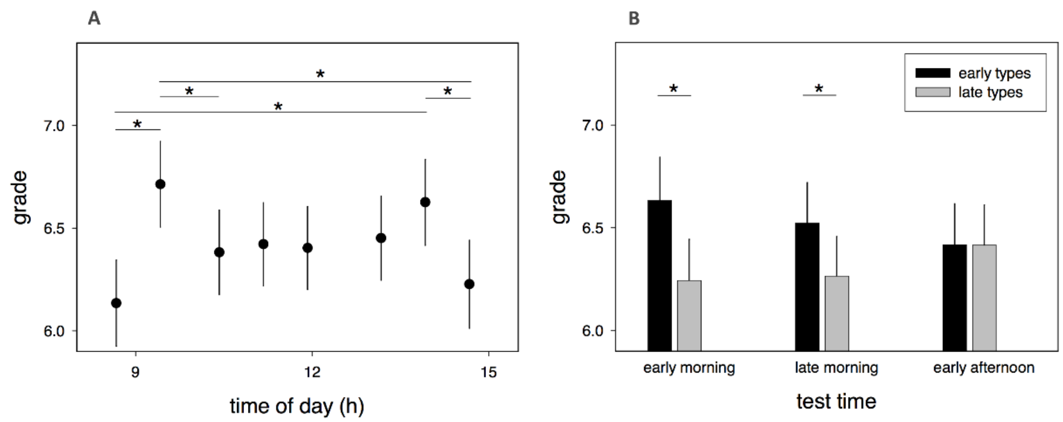

Figure 2. (A) Grades of examinations taken in the 1st and 8th (last) hours were significantly lower compared with grades of examinations taken in the 2nd and 7th hours. (B) The influence of time of day on grades was significantly different between early and late chronotypes. Late types obtained significantly lower grades in the early and late morning compared with early types. This difference disappeared in the early afternoon. Examination grades vary between 1 (lowest) and 10 (highest) with 70% of grades between 5.5 and 7.5; >5.5 represents a passing grade. *p < 0.05

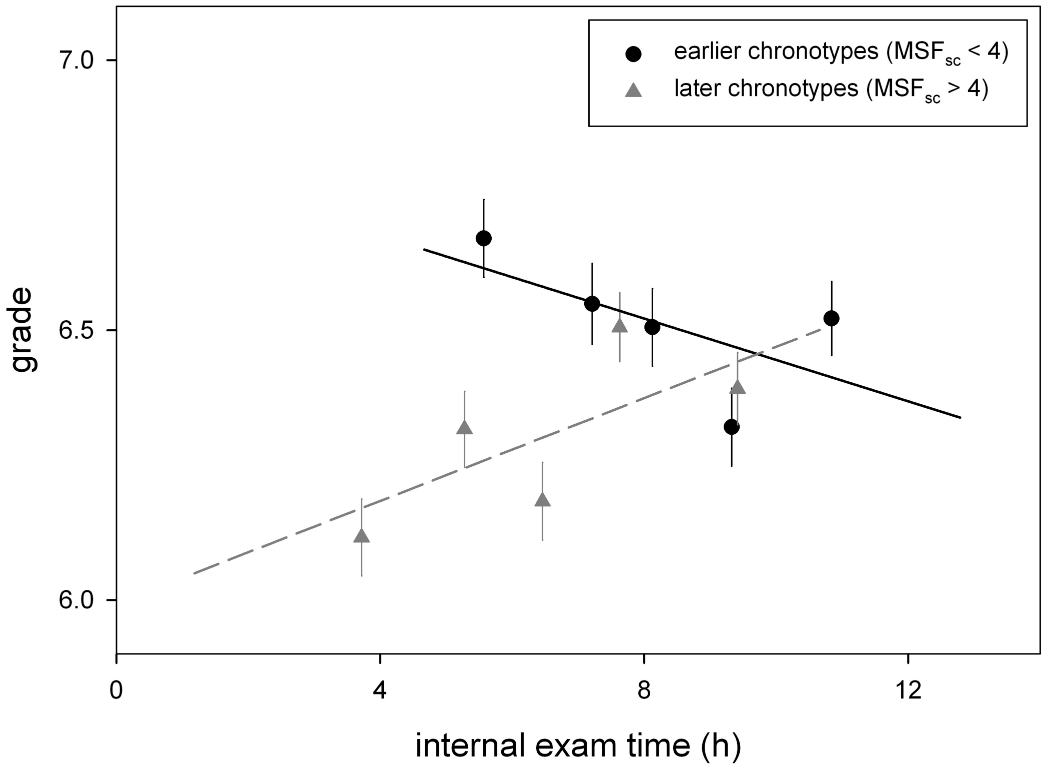

Figure 3. The internal examination time affected school grades differently in early and late chronotypes. The regression lines are based on the analysis of raw examination grades and the associated internal examination time. The range of internal examination times of the raw data is the same as the range covered by the regression lines. The regression analysis is based on the raw data points. The data points summarize average values and SEM for consecutive 20% data subsets per chronotype group. The late-type group had significantly earlier internal examination times compared to the early-type group. The relationship between internal examination time and grade was significantly different in early and late types. Performance of early types decreased while that of late types improved at later internal times. Examination grades vary between 1 (lowest) and 10 (highest) with 70% of grades between 5.5 and 7.5; >5.5 represents a passing grade.

Discussion

Our results show that both short sleep on schooldays and being a late chronotype predict decreased school performance (lower grades). In addition, early and late chronotypes show opposite time-of-day effects on performance. Sleep deficiency is common in adolescents, especially in late chronotypes (Carskadon et al., 1998; Touitou, 2013). Previous studies showed that both sleep deficiency (Meijer, 2008; Perez-Lloret et al., 2013; Wolfson & Carskadon, 2003) and being a late chronotype (Borisenkov et al., 2010) impacts school achievements negatively. However, how time of day alters the relationship between chronotype and academic achievements has received limited attention. Haraszti and colleagues (2014) showed that late chronotypes underperformed early chronotypes only when tested at 08:00 h but not at 14:00 h. Here, we examined the relationships between external time, internal time (chronotype) and performance (examination grades) across a typical Dutch school day, from 08:15 to 15:00 h, showing significant differences between the early and late chronotype groups. While early types performed significantly better in the morning, early and late types performed indistinguishable in the early afternoon. The lowest grades we observed in the first and last (8^{th}) school hours might be a result of additional differential effects of sleepiness in early and late chronotypes. Especially students sleeping fewer than 7 hours per school night had lowest grades, which involved 18% of our participants. This effect, in turn, might be strongest in late chronotypes who – in addition to the short sleep – performed their tests too early in their internal day. The reverse pattern was observed for the early chronotypes, performing worse when tested later in their internal day, which again might result from increased sleepiness in the early types in their last school hour. These findings confirm those of Haraszti et al. (2014). Interestingly, early afternoon often is associated with a 'post-lunch dip' in performance (Bes, Jobert, & Schulz, 2009). However, here we can only speculate that the post-lunch dip might be milder or absent in younger students and/or that it appears at a later time point due to the overall later circadian physiology in adolescents (Carskadon & Dement, 1992; Monk, Buysse, Reynolds, & Kupfer, 1996).

The results of our study add to the accumulating evidence that chronotype should be taken into account in assessments of performance (Borisenkov et al., 2010; Haraszti et al., 2014; Schmidt, Collette, Cajochen, & Peigneux, 2007). In our study, examinations scheduled during the first 2 school hours were taken by the latest chronotypes (MSF_{sc} > 5.31 h) on average 3.1 hours after their MSF_{sc}. Assuming an average of 9 hours of sleep need for most adolescents per night (Owens, Adolescent sleep working group, Committee on adolescence, council on school health, 2014), this finding means that the latest chronotypes took their early school examinations during their biological night. This is supported by a constant routine experiment, showing significant cognitive impairment after awakening during the biological night (Scheer, Shea, Hilton, & Shea, 2008). In addition, beyond its impact on cognitive and academic performance, a mismatch between internal and external time (social jetlag) also significantly compromises health and wellbeing (Kantermann, Wehrens, Ulhôa, Moreno, & Skene, 2012b; Levandovski et al., 2011; Roenneberg, Allebrandt, Merrow, & Vetter, 2012; Wittmann et al., 2006). Our student population on average had 2.3 hours of social jetlag, which is in line with previous studies showing that about 69% of the general working population show at least 1 hour of social jetlag and one third suffer from 2 hours or more (Roenneberg, Kantermann, Juda, Vetter, & Allebrandt, 2013). Albeit not statistically significant, grades in our study were lowest in those students with more than 3 hours of social jetlag, which involved 21.5% of our study population. Therefore, future studies should incorporate the assessment of social jetlag in their study design to explore its impact on school performance in mode detail.

A limitation of our study is the correlational approach, making conclusions regarding causality difficult. This shortcoming could be addressed in future studies, or example, assessing how changing school starting times affects sleep and grades.

In addition, future research should more rigorously control for potential confounders in the assessment of sleep timing, including potential influences of attention-deficity hyperactivity disorder or other attention/learning disorders and also seasonal variations in sleep timing (Allebrandt et al., 2014).

Taken together, our findings emphasize the need for significant amendments to current school legislature. A few schools have managed to implement later school start times and report significant improvements of students' sleep and daytime functioning (Boergers, Gable, & Owens, 2014; Owens, Belon, & Moss, 2010). In addition, tailored interventions to reduce especially short wavelength (blue) light in the evenings and/or to increase light exposure in the mornings could help to synchronize the students' circadian clocks to their school schedules. The circadian clock is most sensitive to short wavelengths (Brainard et al., 2001), and studies have shown that especially blue light from computers and TVs interferes with sleep and the circadian rhythm (van der Lely et al., 2014; Wood, Rea, Plitnick, & Figueiro, 2013). However, such behavioral interventions are as difficult to achieve on a population level, as are changes in school start times. Therefore, as a first step, we suggest a shift of examination schedules to the early afternoon to at least secure equal examination conditions for all chronotypes.

Acknowledgements

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Chapter 3

Lower school performance in late chronotypes: underlying factors and mechanisms

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Abstract

Success at school determines future career opportunities. Earlier, we described a time-of-day specific disparity in school performance between early and late chronotypes. Several additional studies have shown that students with a late chronotype and short sleep duration obtain lower grades, suggesting that early school starting times handicap these students` performance. How chronotype, sleep duration, and time of day impact school performance is not clear. At a Dutch high school, we collected 40,890 grades obtained in a variety of school subjects over an entire school year. We found that the strength of the effect of chronotype on grades was similar to that of absenteeism, and that late chronotypes were more often absent. The difference in grades between the earliest 20% and the latest 20% of chronotypes corresponds to a drop from the 55th to 43rd percentile of grades. In academic subjects using mainly fluid cognition (scientific subjects), the correlation with grades and chronotype was significant while subjects relying on so-called crystallized intelligence (humanistic/linguistic) showed no correlation with chronotype. Based on these and previous results, we can expand our earlier findings concerning exam times: students with a late chronotype are at a disadvantage in exams on scientific subjects, and when they are examined early in the day.

Introduction

The gateway to success is education. What pupils learn and how they perform during primary and secondary education influences their future career opportunities (French, Homer, Popovici, & Robins, 2015). Academic beliefs (e.g. perceived academic competence), motivation, and intelligence have been shown to play an important role in school performance (Deary, Strand, Smith, & Fernandes, 2007; Fortier, Vallerand, & Guay, 1995). Other factors related to class and family environment such as teacher quality, socio-economic status, and parental involvement are also associated with school achievements (Juang & Silbereisen, 2002; Pokropek, Borgonovi, & Jakubowski, 2015; Rockoff, 2004).
The role of sleep in relation to school performance has been extensively studied. Cognitive performance can be quantitatively impaired by sleep deprivation and high-school students usually carry more sleep debt than younger or older individuals (Dinges, Pack, Williams, & Gillen, 1997; Hagenauer, Perryman, Lee, & Carskadon, 2009; Lo et al., 2012). Previously, we reported that students who are late chronotypes – those who sleep at the latest times of the day – perform worse on exams that are scheduled in the morning in comparison to those scheduled later in the day(van der Vinne et al., 2015). Importantly, early and late chronotypes in our study performed equally well in the afternoon. A number of reports have purported that either early or late chronotypes are more or less intelligent (Arbabi, Vollmer, Dörfler, & Randler, 2014; Goldstein, Hahn, Hasher, Wiprzycka, & Zelazo, 2007; Piffer, Ponzi, Sapienza, Zingales, & Maestripieri, 2014). Based on the lack of agreement between these studies, their weak significance, and our previous findings, we assume that chronotype is not associated with intelligence. Chronotype can be assessed via the Munich ChronoType Questionnaire (MCTQ; Roenneberg, Wirz-Justice, & Merrow, 2003) as the midpoint of sleep on work-free days (MSF). This value is further corrected for sleep debt accumulated on school/work days (MSF_{sc}). Chronotype is predominantly controlled by the circadian clock and external timing signals (zeitgebers) (Roenneberg et al., 2003). Humans entrain (synchronize) with different phases to the external light-dark cycle, giving rise to a distribution of chronotypes, ranging from early (larks) to late (owls) types (Roenneberg & Merrow, 2007). Chronotype varies with age and is latest during adolescence (Crowley et al., 2014; Roenneberg et al., 2004). Despite the late chronotype in adolescents, several schools start early in the morning (8:30 h on average in the Netherlands), leading to chronic sleep deprivation in most high-school students (C. E. Basch, Basch, Ruggles, & Rajan, 2014; Gibson et al., 2006).
Late chronotype has been correlated with shorter sleep duration on school/work days (Roenneberg et al., 2007), and late types as well as short sleepers have been shown to obtain lower grades on average (Borisenkov, Perminova, & Kosova, 2010; van der Vinne et al., 2015).

The influence of chronotype, sleep duration, and time of day on school performance has received some attention in previous studies. One possibility is that late chronotypes are tested at an earlier internal time (internal time can be expressed as hours since MSF_{sc}) before they reach their peak performance. This is supported by our previous finding that the chronotype-effect on grades is pronounced in the early morning, but insignificant in the early afternoon (van der Vinne et al., 2015). Highly controlled laboratory experiments have found that cognitive abilities relying mainly on so-called fluid intelligence (e.g. logic, reasoning, problem solving) are susceptible to time-of-day and chronotype-effects (Fimm, Brand, & Spijkers, 2015). Early chronotypes tend to perform better in the morning while late chronotypes perform better in the evening (Goldstein et al., 2007; Lara, Madrid, & Correa, 2014). Crystallized intelligence (e.g. general knowledge, long-term memory vocabulary), on the contrary, was found to be immune to time-of-day and chronotype-effects (Barbosa & Albuquerque, 2008; Folkard & Monk, 1980).

Another possible explanation for lower school performance in late chronotypes is that chronic sleep deprivation impairs cognitive abilities. Sleep deprivation can affect functioning of the prefrontal cortex and cortical-thalamic circuits, which are involved in controlling high-order cognitive functions, such as logic and reasoning, abstract thinking, and problem solving (fluid intelligence) (Cajochen, Foy, & Dijk, 1999; Thomas et al., 2000). Although sleep supports memory consolidation, access to long-term-acquired knowledge (crystallized intelligence) seems to be less impaired by sleep deprivation compared to fluid intelligence (Alhola & Polo-Kantola, 2007; Gais, Lucas, & Born, 2006; Randazzo, Muehlbach, Schweitzer, & Walsh, 1998).

Chronotype could also be associated with other factors (e.g. school attendance) involved in determining school achievements. Absenteeism was found to correlate negatively with worse grades (Roby, 2004), but research on the relationship between chronotype and school attendance/absenteeism is lacking. Early school starting times challenge late chronotypes more than early chronotypes, which could lead to more tardiness (e.g. due to oversleep), and more days of sick leave in late chronotypes with negative consequences for their school grades.

The aim of the current study is to explore if chronotype, sleep duration on school nights, and school attendance alone and in combination can predict school performance. We analyzed grades obtained in Dutch high-school students over an entire school year. When considering this specific set of predictors of school performance, we found that chronotype had a stronger impact on grades than sleep duration. This association was strongest for scientific subjects. Absenteeism was increased in late chronotypes and was associated with an overall decrease in grades, independent of school subject.

Methods

Study protocol

The study was conducted at a Dutch high school in Coevorden (52° 40' N / 6° 45' E) between August 2013 and July 2014. The secondary education in the Netherlands is organized in three levels: the VMBO (voorbereidend middelbaar beroepsonderwijs) prepares students for the job market (4 years of education from age 12 to 16); the HAVO (hoger algemeen voortgezet onderwijs) prepares students to study at universities of applied sciences (5 years of education from age 12 to 17); the VWO level (voorbereidend wetenschappelijk onderwijs) prepares students to study at research universities (6 years of education from age 12 to 18). We collected 40,890 school grades in 523 students attending the first three years of secondary school. Between September and November 2013, 426 students filled in the Munich ChronoType Questionnaire (MCTQ; Roenneberg et al., 2003). Chronotype was determined (mid-point of sleep on school-free days corrected for sleep debt on school days; MSF_{sc}). The MCTQ also allows assessing other sleep-related variables, such as average sleep duration of the week, sleep duration separately on school days and school-free days, and social jetlag. The latter is an approximate quantification of the mismatch between the biological and social clocks (Wittmann, Dinich, Merrow, & Roenneberg, 2006).

The school subjects assessed in this study were geography, history, Dutch, English, biology, mathematics, chemistry, and physics. In the Dutch secondary school system, grades range from 1 (lowest) to 10 (highest), with 6 considered to be the threshold to pass an examination (International Recognition Department of Nuffic Netherlands Organisation for International Cooperation in Higher Education, 2013). Grades were collected during 4 periods (Fall: August - October; Winter: November - January; Spring: February - April; Summer: May - July). Students from a total of 20 classes participated in the study. These spanned two levels: the HAVO and the VWO. 12 of the 20 classes belonged to the HAVO, and 8 classes were drawn from the VWO. An overview of all classes by level and by year of education is reported in the Supplementary Table S1. This hierarchy in school levels was mirrored in our analysis using a multilevel approach with students nested within classes, and classes nested within levels of education. Late arrivals, dismissals from class (when a student due to misbehavior was sent out of class by the teacher), frequency of sick leaves and duration of each sick leave in days were extracted from the school's registration system.

Statistical analysis

Statistical analyses were done using R software version 3.3.0 (The R Core team, 2013). A multilevel approach was used to explain the effects of the independent variables on school grades (dependent variable). The independent variables assessed were: demographic variables (sex and age), school attendance variables (late arrivals during the first hour, dismissals from class at any time of day, sick leaves, and sick leave duration), and sleep-related variables (chronotype; MSF_{sc}), social jetlag, and sleep duration on school days. We built nine multilevel models, each with a different subset of explanatory variables. Student ID was included as a random factor nested within class and within level of education (HAVO and VWO). School subject (geography, history, Dutch, English, biology, mathematics, chemistry, and physics) and time of year/season when the grades were collected (Fall: August - October; Winter: November - January; Spring: February - April; Summer: May - July) were entered in all models, and analyzed as covariates. Model selection based on the Akaike Information Criterion (AIC; Akaike, 1973) was performed to select the best combination (fit) of independent variables explaining the variation in school grades. The most parsimonious model is defined as the model with the lowest AIC value. We used the guidelines of Kass and Raftery to compare models (Kass & Raftery, 1995). The estimates of the model are indicated in the results as "b" coefficients. To compare the strength of the effects of the different predictors the coefficients were standardized and are indicated as "β" coefficients.

To further explore the relationship between chronotype, school attendance, sleep duration, and school performance, we ran 4 separate mediation analyses with chronotype as predictor, and late arrivals, dismissals from class, sick leaves, and sleep duration on school days as mediators. Average grades over the entire school year were used as the dependent variable. Sex and class were analyzed in these models as covariates. General linear models with Poisson regression were used to analyze the relationship between chronotype and school attendance variables. Linear models were used to analyze the relationship between chronotype and sleep duration, and between chronotype and average grades. Analyses were done using the R package for causal mediation analysis (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014).

The relationship between chronotype and school attendance variables was modeled taking into account the distribution of data for the specific variables of interests (late arrivals, dismissals from class, sick leaves and sick leave duration). Zero inflation models (to account for the high frequency of zero values) with negative binomial distribution (to account for over-dispersion of the data) were chosen to model the effect of chronotype on late arrivals and dismissals from class. Zero inflation models combine two processes: the first model predicts whether an event has occurred or not and is governed by a binary distribution; the second model predicts how many times an event is likely to occur and is governed by a Poisson distribution (count data). General linear models with quasi-Poisson regression were used to test the effect of chronotype on frequency and duration of sick leaves. Sex and age were added to the models as covariates. Model selection based on AIC was again applied.

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 Centre of Groningen (NL) and the head of the school approved the study. Written informed consent was obtained from the head of the school.

Results

A total of 40,890 grades from individual examinations taken by students attending the first three years of secondary education (523 students; average number of grades per student: 78; age range: 11-17 years) were collected. Of these students, 426 (219 females and 207 males, mean age 13.06 ± 0.95 SD; age range 11-16 years) had filled in the MCTQ to assess their chronotype, social jetlag, and average sleep duration on school nights (Table 1 and Supplementary Fig. S1). In the Dutch secondary school system, grades range from 1 (lowest) to 10 (highest), with 6 considered to be the threshold to pass an examination (International Recognition Department of Nuffic Netherlands Organisation for International Cooperation in Higher Education, 2013). The conversion between Dutch grades and US grades is the following: 1-5 = F, 5.5 = D, 6 = C, 6.5 = B, 7 = B+, 7.5-8 = A, 8.5-10 = A+.

Table 1. Demographics of 219 female and 207 male high school students attending the first three school years (data from the Munich ChronoType Questionnaire).

Outcome measure	Average (± SD)	Range
Age (years)	13.06 (0.95)	11 - 16
Chronotype (MSF_{sc}, h)	4.26 (1.08)	-0.38 - 8.38
Social Jetlag (h)	2.21 (0.98)	0 - 5.94
Sleep onset on school days (h)	-1.39 (0.98)	-3.42 - 4.00
Sleep end on school days (h)	6.63 (0.42)	4.50 - 8.25
Sleep duration on school days (h)	8.02 (1.05)	3.25 - 10.67
Sleep onset on school-free days (h)	0.09 (1.35)	-2.83 - 5.25
Sleep end on school-free days (h)	9.53 (1.47)	2 - 14
Sleep duration on school-free days (h)	9.44 (1.47)	1.5 - 12.67

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).

Data on school attendance were retrieved from the school's registration system. The number and percentage of students absent from class together with the total counts of late arrivals, dismissals from class, sick leaves and duration of sick leaves are reported in Table 2.

The influence of the explanatory variables (demographic, sleep-related, and school attendance variables) on school grades was assessed with a multilevel approach. Our analysis of model selection based on the AIC indicated that model 1 and model 4 (AIC model 1: 118909.8; AIC model 4: 118909) were the most parsimonious models to explain the variation in school grades. Both models had chronotype, sex, late arrivals during the first hour, dismissals from class, and sick leaves (duration) as predictors, and model 4 had age as additional predictor. Since age was found not to be significantly associated with grades, we report here the results obtained with model 1, following the principle of parsimony (Vandekerckhove, Matzke, & Wagenmakers, 2014) (Fig. 1; see Supplementary Table S2 for a detailed description of the 9 models, and the comparison of AIC values between models).

Table 2. Number and percentage (%) of students absent from class.

	All students		Students with MCTQ	
Outcome measure	N students (%)	Count	N students (%)	Count
Late arrivals	249 (47%)	589	197 (46%)	421
Dismissals from class	153 (29%)	353	120 (28%)	272
Sick leaves	395 (75%)	1,346	316 (74%)	1,025
Sick leave duration (d)	395 (75%)	2,259	316 (74%)	1,627

Number and percentage of students are reported separately for the entire student population (523), and for the students who filled in the MCTQ (426). The total counts of late arrivals, dismissals from class, sick leaves, and sick leave duration (days) are also reported.

We found that chronotype was negatively correlated with grades, with later chronotypes obtaining lower grades compared to earlier chronotypes ($b = -0.060$, $t (407) = -2.313$, $p = 0.0212$). A one-hour later chronotype correlated with an overall decrease in grades with a factor of 0.06 (on a scale from 1 to 10). Sex also had a significant effect on grades ($b = -0.138$, $t (407) = -2.542$, $p = 0.0114$), with males obtaining lower grades (on average 0.14 lower) compared to females. School attendance was found to be associated with grades, with increased absenteeism negatively impacting grades (late arrivals: $b = -0.062$, $t (407) = -3.283$, $p = 0.0011$; dismissals from class: $b = -0.090$, $t (407) = -4.608$, $p < .0001$; sick leave duration: $b = -0.019$, $t (407) = -3.875$, $p = 0.0001$).

Our model predicted an overall decrease in grades of 0.09 for a student dismissed from class one time, a decrease of 0.06 for a student arriving late one time, and a decrease of 0.02 for a student being sick one day in the course of an entire school year. To compare the strength of the effects of this set of predictors, we calculated the standardized beta (β) coefficients (Fig. 1). The effect on grades was stronger for dismissals from class ($\beta = -0.087$), followed by sick leave duration ($\beta = -0.065$), late arrivals ($\beta = -0.061$), sex ($\beta = -0.044$), and chronotype ($\beta = -0.042$).

Short sleep duration was found to be significantly associated with lower grades only in model 9 ($b = 0.083$, $t (423) = 2.943$, $p = 0.0034$). This model did not include the school attendance variables as predictors and had the highest AIC value (worse fit) among all models considered. Similarly, increased levels of social jetlag were significantly associated with lower grades only in model 8 ($b = -0.094$, $t (409) = -3.188$, $p = 0.0015$), which did not include the school attendance variables as predictors.

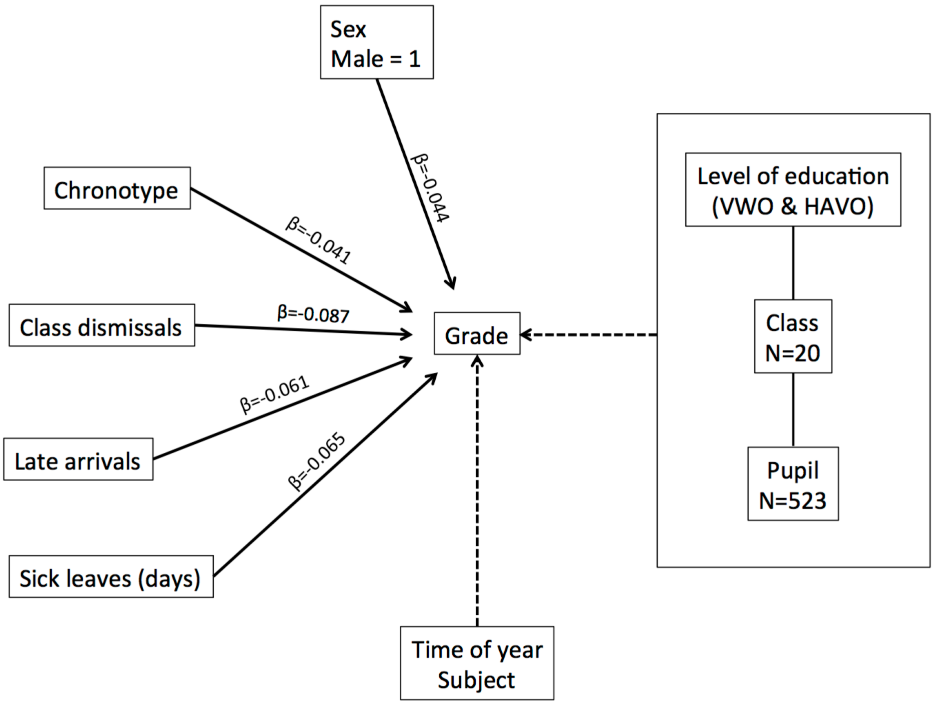

Figure 1. Multilevel model selected as the most parsimonious fit (according to the AIC) to explain the influence of the independent variables on school grades (dependent variable). The explanatory (independent) variables were: sex, chronotype (MSF_{sc}), late arrivals during the first hour, class dismissals, and sick leaves (duration). The standardized beta coefficients (β) were negative for each variable and are reported on the solid connecting lines between independent and dependent variables. The interpretation of a negative beta coefficient is the following: for every 1-standard deviation increase in the explanatory variable, the standard deviation of the dependent variable will decrease by the beta coefficient value. For the variable 'sex', males were compared with females, meaning that a negative beta coefficient reflected a decrease in grades for males. Time of year and school subject were evaluated in the model as covariates (dashed connecting lines).

The mediation analysis showed that the direct effect of chronotype on grades was significant (late arrivals: b = -0.085, p = .01; dismissals from class: b = -0.071, p = .01; sick leave (duration): b = -0.087, p < .01; sleep duration on school days: b = -0.081, p < .01), while the indirect effect of chronotype mediated by late arrivals, dismissals from class, sick leave (duration), and sleep duration on school days was not (late arrivals: b = -0.005, p = 0.32; dismissals from class: b = -0.006, p = 0.15; sick leave (duration): b = -0.005, p=0.12; sleep duration on school days: b = -0.015, p = 0.09).

In addition to the influence on grades, we found that chronotype was related to school attendance (Fig. 2a-2d). Chronotype influenced the likelihood of arriving late to the first lesson of the day (b = -0.695, z = -2.555, p = 0.0106; Fig. 2a). For instance, a student with a chronotype of 3 had two times larger odds of never being late compared to a student with a chronotype of 4 (MSF_{sc} of one hour later). Age influenced the frequency of late arrivals, with older students arriving late more often compared to younger students (b = 0.320, z = 4.700, p < .0001). Later chronotypes and older students were more likely to be dismissed from class (chronotype: b = -0.928, z = -2.285, p = 0.0223; Fig. 2b; age: b = -1.498, z = -2.568, p = 0.0102).

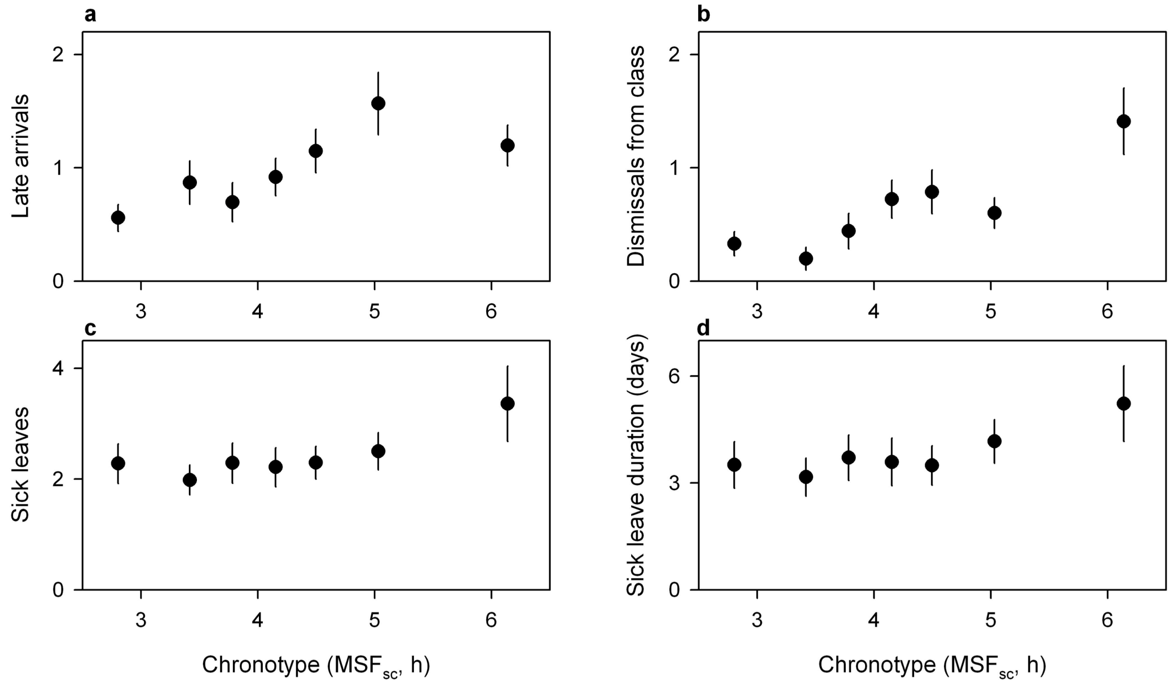

Figure 2. Influence of chronotype (MSF_{sc}) on school attendance.
Data points represent mean number of late arrivals (a), dismissals from class (b), sick leaves (c) and days of sick leave (d) with standard error of the mean (SEM). The averages were calculated over the entire school year. The students were divided into 7 equal-sized groups based on chronotype (lower numbers correspond to earlier chronotypes and higher numbers to later chronotypes, respectively). Late chronotypes were significantly more likely to arrive late, be dismissed from class, become sick, and miss more days due to sickness.

Among the students who had been dismissed from class at least once per school year, younger and male students had an increased chance of being dismissed more often (age: b = -0.391, z = -2.685, p = 0.0073; sex: b = 0.831, z = 3.447, p = 0.0006). Chronotype also influenced the frequency and the duration of sick leaves, with late chronotypes being more often and more days sick (sick leave frequency: b = 0.125, t (420) = 2.202, p = 0.0282; Fig. 2c; sick leave duration: b = 0.123, t (420) = 1.975, p = 0.049; Fig. 2d). We did not find a significant effect of sex and age on the number of sick leaves. The complete overview of the effects of chronotype, sex, and age on school attendance is reported in Table 3.

Table 3. Influence of chronotype, sex, and age on school attendance variables.

	late arrivals (zero-inflation model)			class dismissals (zero-inflation model)		
explanatory variables	b	z-value	p-value	b	z-value	p-value
chronotype	-0.695	-2.555	0.0106	-0.928	-2.285	0.0223
sex	---	---	---	-0.990	-1.388	0.1651
age	---	---	---	-1.498	-2.568	0.0102

	late arrivals (count model)			class dismissals (count model)		
explanatory variables	b	z-value	p-value	b	z-value	p-value
chronotype	---	---	---	0.192	1.796	0.0725
sex	---	---	---	0.831	3.447	0.0006
age	0.320	4.700	<.0001	-0.391	-2.685	0.0073

	sick leaves			sick leaves (days)		
explanatory variables	b	t-value	p-value	b	t-value	p-value
chronotype	0.125	2.202	0.0282	0.123	1.975	0.049
sex	-0.224	-1.839	0.0666	-0.217	-1.620	0.106
age	0.064	0.984	0.3258	0.101	1.413	0.158

The model estimates (b coefficients), the test statistic (z-test and t-test), and the p-values associated with each independent variable are reported. For late arrivals and class dismissals both the zero-inflation model and the count model statistics are indicated. If a variable was not included in the final selected model, this is indicated by horizontal dashes (---).

Finally, we added an interaction effect (chronotype x school subject) to our multilevel model to explore whether the size and significance of effect of chronotype on grades was different between school subjects. We found that the interaction effect was significant ($F_{7,32920}$ = 15.490, $p < .0001$), meaning that the slopes of the regression lines describing the effect of chronotype on grades were significantly different between school subjects. We therefore fitted the multilevel model separately by school subject (Fig. 3).

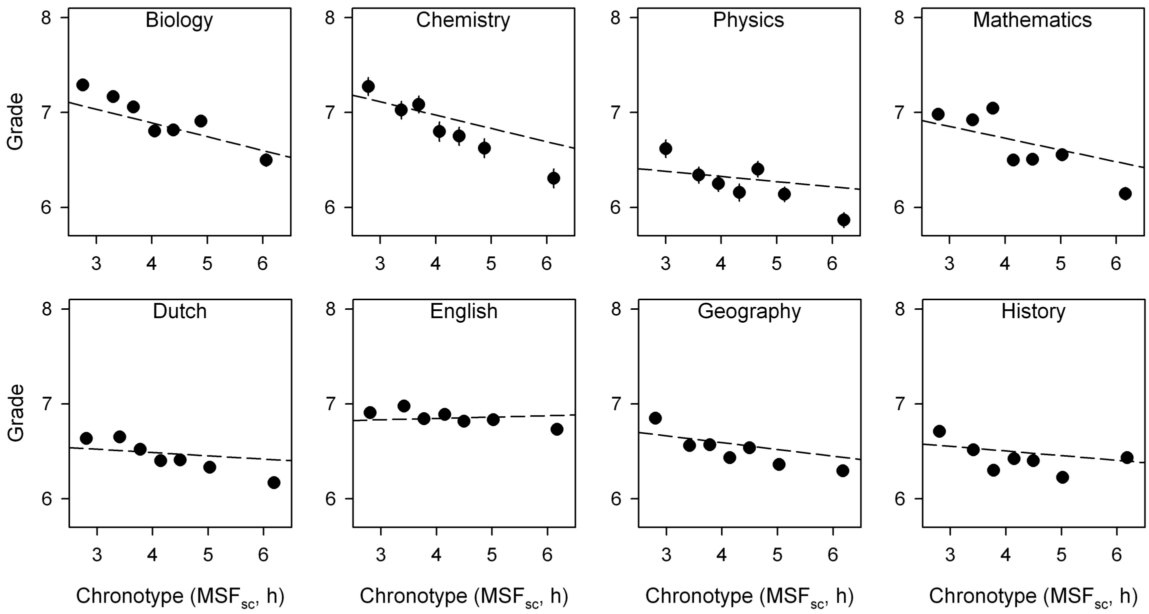

Figure 3. Influence of chronotype (MSF_{sc}) on grades by subject.
Data points represent mean grades with standard error of the mean (SEM). Since the SEM were very small, error bars are not always visible. Mean grades were calculated for 7 equal-sized groups of students based on chronotype (lower numbers correspond to earlier chronotypes and higher numbers to later chronotypes, respectively). Regression lines representing the association between chronotype and grades (raw data) were calculated with multilevel mixed modeling separately per each subject. The raw data are shown in Supplementary Figure S2. The influence of chronotype on grades was significant only for geography, biology, chemistry, and mathematics.

The effect of chronotype on grades was significant for geography (b = -0.071, t (405) = -2.559, p = 0.0108), biology (b = -0.145, t (254) = -3.423, p = 0.0007), chemistry (b = -0.141, t (162) = -2.412, p = 0.0170), and mathematics (b = -0.124, t (405) = -2.543, p = 0.0114), and was not significant for English (b = 0.014, t (405) = 0.315, p = 0.7528), history (b = -0.050, t (405) = -1.316, p = 0.1889), physics (b = -0.058, t (99) = -0.738, p = 0.4621), and Dutch (b = -0.034, t (405) = -1.414, p = 0.1581). In geography, biology, chemistry, and mathematics late chronotypes obtained lower grades compared to early chronotypes. The complete overview of the effects of all variables on grades by subject is reported in Table 4. Based on these results, we divided the school subjects into two groups: scientific (biology, physics, chemistry, and mathematics) and humanistic/linguistic subjects (Dutch, English, history, and geography). The interaction effect between chronotype and subject area (scientific vs. humanistic/linguistic) was significant (Fig. 4; $F_{1,32932} = 73.567$, $p < .0001$), with the effect of chronotype on grades being significantly stronger for scientific subjects compared to humanistic/linguistic subjects.

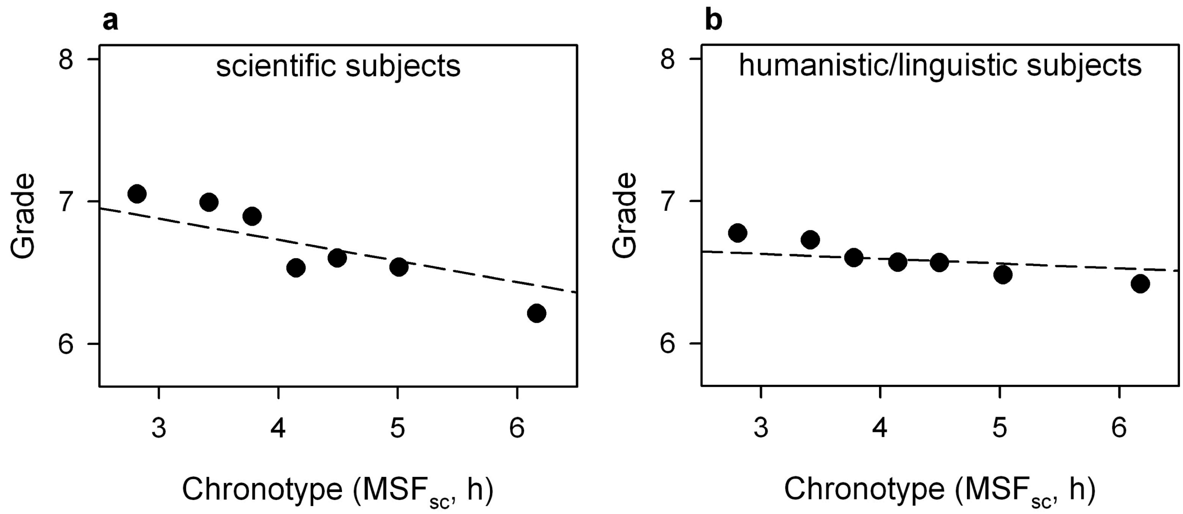

Figure 4. Influence of chronotype (MSF$_{sc}$) on grades by subject area.
Data points represent mean grades with standard error of the mean (SEM). Since the SEM were very small, error bars are not always visible. Mean grades were calculated for 7 equal-sized groups of students based on chronotype (lower numbers correspond to earlier chronotypes and higher numbers to later chronotypes, respectively). Regression lines representing the association between chronotype and grades (raw data) were calculated with multilevel mixed modeling separately per each subject. The raw data are shown in Supplementary Figure S3. The influence of chronotype on grades was significantly stronger for scientific subjects (biology, chemistry, physics, and mathematics) (a) compared with humanistic/linguistic subjects (Dutch, English, geography, and history) (b).

Table 4. Influence of demographic, sleep-related and school attendance variables on school grades by academic subject.

	geography			history			English			Dutch		
explanatory variables	b	t-value	p-value	b	t-value	p-value	b	t-value	p-value	b	t-value	p-value
sex	0.125	2.125	0.0342	0.132	1.651	0.0995	-0.274	-2.857	0.0045	-0.326	-6.446	< .0001
chronotype	-0.071	-2.559	0.0108	-0.049	-1.316	0.1889	0.014	0.315	0.7528	-0.034	-1.414	0.1581
late arrivals	-0.052	-2.489	0.0132	-0.088	-3.299	0.0011	-0.070	-2.066	0.0395	-0.048	-2.685	0.0075
class dismissals	-0.076	-3.575	0.0004	-0.047	-1.656	0.0985	-0.067	-1.953	0.0515	-0.101	-5.545	< .0001
sick leaves (days)	-0.014	-2.723	0.0067	-0.022	-3.097	0.0021	-0.012	-1.408	0.1598	-0.014	-3.175	0.0016

	biology			physics			chemistry			mathematics		
explanatory variables	b	t-value	p-value	b	t-value	p-value	b	t-value	p-value	b	t-value	p-value
sex	-0.116	-1.349	0.1787	0.082	0.512	0.6099	0.249	2.164	0.0319	-0.188	-1.823	0.0690
chronotype	-0.145	-3.423	0.0007	-0.058	-0.738	0.4621	-0.141	-2.412	0.0170	-0.124	-2.543	0.0114
late arrivals	-0.062	-1.939	0.0536	0.020	0.510	0.6114	-0.093	-2.074	0.0397	-0.065	-1.846	0.0656
class dismissals	-0.079	-2.152	0.0323	-0.256	-3.313	0.0013	-0.101	-3.018	0.0030	-0.126	-3.435	0.0007
sick leaves (days)	-0.031	-3.910	0.0001	-0.018	-1.525	0.1303	-0.034	-9.296	0.0012	-0.031	-3.290	0.0011

The model estimates (b coefficients), the test statistic (t-test), and the p-values associated with each independent variable are reported.

Discussion

The aim of the current study was to quantify the impact of chronotype, sleep duration, time of day, and school attendance on school performance. We collected grades and school attendance data in Dutch high-school students over an entire school year. Our results are consistent with other studies, in that chronotype correlates significantly with school grades: late chronotypes obtain, on average, lower grades (Borisenkov et al., 2010; Escribano, Díaz-Morales, Delgado, & Collado, 2012; Preckel et al., 2013; van der Vinne et al., 2015; Vollmer, Pötsch, & Randler, 2013). The strength of the chronotype effect was comparable to the negative effect of absenteeism on grades. A one-hour later chronotype was associated with a decrease in grades with a factor of 0.06 (on a scale from 1 to 10). With a difference of almost 3 hours between the earliest 20% (mean MSF_{sc} = 2:56 h) and the latest 20% (mean MSF_{sc} = 5:53 h) of chronotypes in our sample, the model predicted an overall decrease in grades of 0.18 for late compared to early chronotypes. This represents a difference from the 55th to the 43rd percentile of grades in our data set.

The model selection based on the AIC revealed that sleep duration was one of the weaker predictors among our explanatory variables. Rather chronotype, sex, and school attendance most closely correlated with school grades. Sleep duration was significantly associated with grades only without controlling for other variables such as school attendance and chronotype. In addition, our mediation analysis revealed a significant direct effect of chronotype on grades independent of sleep duration on school days. Comparison between the direct and the indirect (sleep-duration mediated) effect of chronotype showed that the strength of the effect of the former was 5 times larger. Although several reviews have reported an association between short sleep duration and poor school performance (Curcio, Ferrara, & De Gennaro, 2006; Dewald, Meijer, Oort, Kerkhof, & Bögels, 2010; Taras & Potts-Datema, 2005; Wolfson & Carskadon, 2003), our findings suggest that chronotype has a stronger influence on school performance. Since a late chronotype can lead to short sleep duration on school/work days (Roenneberg et al., 2007), our work emphasizes the need to disentangle these variables in future studies. It is important to mention that we did not collect data about napping behavior in these students. It is possible that the effect of sleep duration on grades was not evident because some students compensated their daily sleep debt through napping.
Similarly to sleep duration, levels of social jetlag were found to be associated with grades only when not statistically controlling for school attendance. Previous studies have shown a relationship between increased levels of social jetlag and lower school/academic performance (Díaz-Morales & Escribano, 2015; Haraszti, Ella, Gyöngyösi, Roenneberg, & Káldi, 2014). However, our findings indicate that chronotype was a stronger predictor of school performance than social jetlag.

In addition, we found that chronotype was significantly associated with school attendance, suggesting that the effect of chronotype on grades could be mediated by school attendance: late chronotypes are more often absent, absenteeism is related to lower school performance, and therefore late chronotypes obtain lower grades. However, the results from the mediation analysis indicated that chronotype had a direct effect on grades independent of school attendance. The direct effect of chronotype on grades was on average 15 times larger than the indirect effect of chronotype on grades mediated by school attendance.

Our analyses showed that the effect of chronotype on grades was only significant for scientific subjects (except for physics) and not for humanistic/linguistic subjects. The absence of an effect in physics might be the result of the smaller number of grades present in the dataset for this subject. This is supported by a similar negative estimate for physics as for the other scientific subjects. In contrast to chronotype, absenteeism was always significantly associated with grades obtained in every school subject. Based on these results, we hypothesize that chronotype and school attendance impact school performance differently. On one hand, absenteeism is likely to impair school performance when a student is learning for all subjects that had been taught while he/she was absent, resulting in lower grades independent of subject. Chronotype, on the other hand, may impact specific cognitive processes that are important for scientific subjects, resulting in lower grades only for these subjects. This hypothesis is supported by previous research showing that both chronotype and time of day have a stronger effect on cognitive performance in tasks requiring fluid intelligence (reasoning, logic, abstract thinking) than on those using crystallized intelligence (general knowledge) (Barbosa & Albuquerque, 2008; Fimm et al., 2015; Folkard & Monk, 1980; Goldstein et al., 2007; Lara et al., 2014). Fluid intelligence, in turn, characterizes thought processes used for exams in scientific subjects rather than humanistic/linguistic subjects (Chapelle & Green, 1992; Primi, Ferrão, & Almeida, 2010). Studies measuring brain activity with EEG and fMRI also found chronotype and time-of-day variation on tasks involving fluid intelligence (Huang, Katsuura, Shimomura, & Iwanaga, 2006; Schmidt et al., 2012).

In contrast to our results, a recent study assessing the effect of diurnal preferences on different school subjects found that eveningness (but not morningness) was negatively associated with grades obtained in both scientific and linguistic subjects (Preckel et al., 2013). However, both the assessment of chronotype (two-dimensional) and grades (self-reported grades averaged per subject area) used different methods compared to our study, limiting a direct comparison between studies.

For our work, we used an approach based on regression analysis. It is important to note that this, per definition, does not allow investigating causal relationships among variables of interest. In addition, we analyzed a specific set of predictors of school performance, and we did not assess other factors, such as academic beliefs and academic motivation, that have been previously found to be associated with school grades (Deary et al., 2007; Fortier et al., 1995). Future studies may therefore include some of these factors. For instance, achievement motivation has been found to mediate the effect of chronotype on grades (Arbabi et al., 2014; Roeser, Schlarb, & Kübler, 2013).

Because of the consistent chronotype-effect on grades described in many studies, future research should focus on investigating the mechanisms underlying this effect so that evidence-based school policies can be implemented. As it stands now, early school starting times are a form of discrimination against late chronotypes, neglecting the potential benefits of making full use of the reach variety in this biological trait (Arbabi et al., 2014; Borisenkov et al., 2010; Escribano et al., 2012; Preckel et al., 2013; Randler & Frech, 2006; van der Vinne et al., 2015; Vollmer et al., 2013). Compared to other factors (socio-economic status, academic beliefs, intelligence), adaptation of school schedules to the students' sleep needs is relatively easy – and the payoff is high.

Taken together our findings suggest that a change in school schedules would improve school attendance and performance, especially in late chronotypes. There is growing evidence that schools start too early for the circadian clocks of adolescents (C. E. Basch et al., 2014; Carskadon, Wolfson, Acebo, Tzischinsky, & Seifer, 1998; Roenneberg et al., 2004; van der Vinne et al., 2015). More field studies investigating the impact of delayed school starting times are needed, but the first findings are promising in terms of improved students' sleep, mood, behavior, school attendance, and performance (Boergers, Gable, & Owens, 2014; Carrell, Maghakian, & West, 2011; Owens, Belon, & Moss, 2010; Thacher & Onyper, 2016). Our current and previous findings also suggest additional solutions: school schedules could be adapted, favoring examinations later in the day, especially for scientific subjects.

Acknowledgments

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Supplementary Information

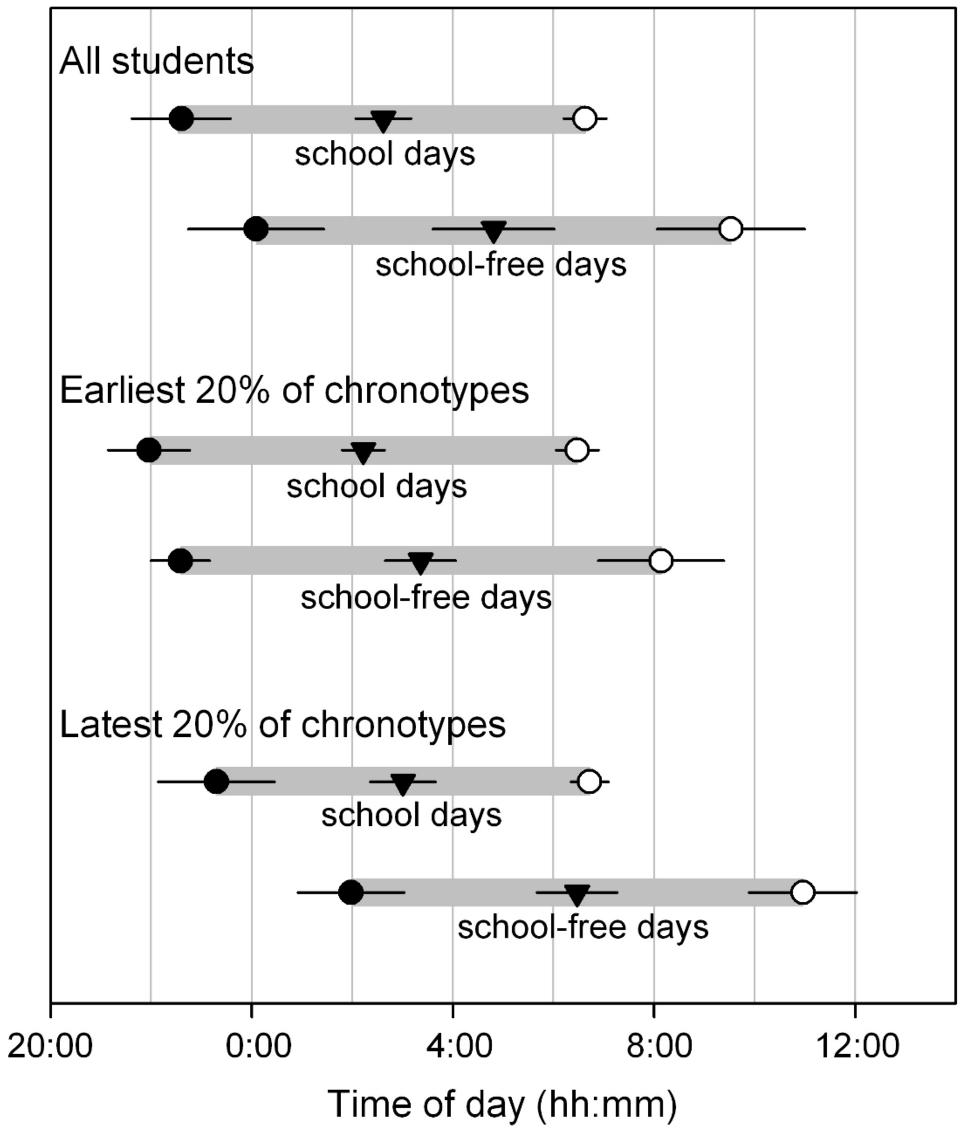
Supplementary Table S1. Overview of the classes by level and by school year of education.

	HAVO	VWO
1st year	1h1, 1h2, 1h3, 1h4	1a1, 1a2, 1g1
2nd year	2h1, 2h2, 2h3, 2h4	2a1, 2a2, 2g2
3rd year	3h1, 3h2, 3h3, 3h4	3a1, 3ag1

Supplementary Table S2. Description of the 9 models used to explore the influence of demographic, sleep-related, and school attendance variables on school grades.

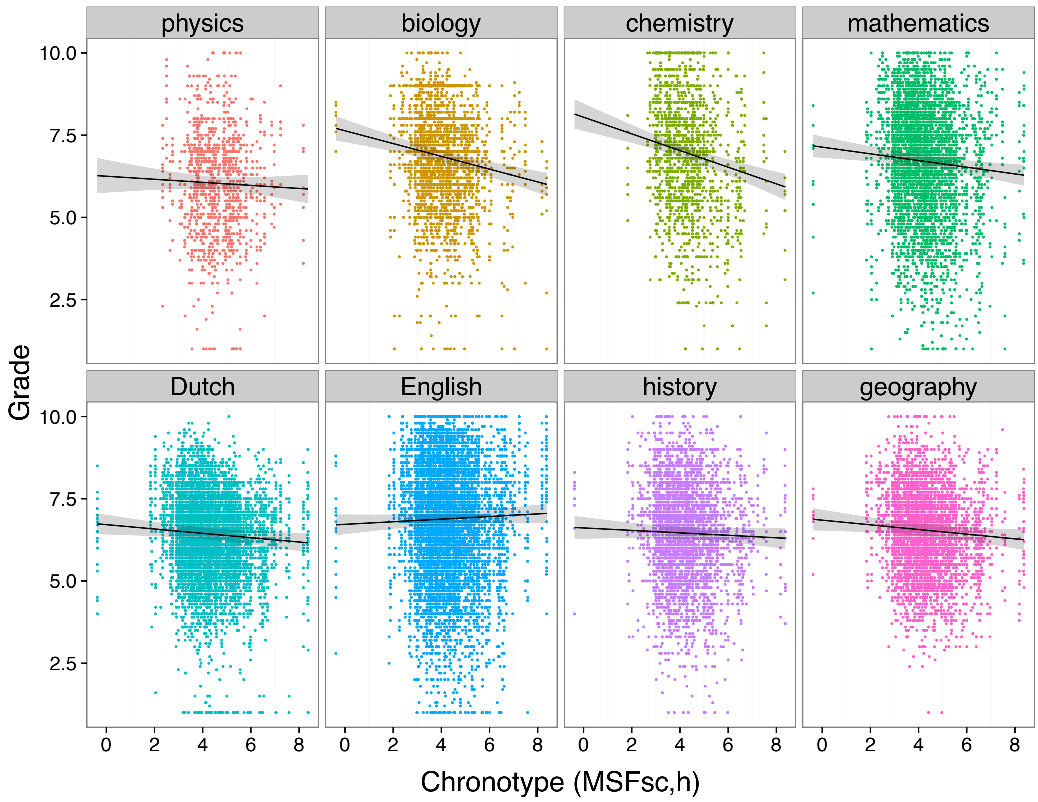
Model	Explanatory variables	K	AICc	Delta AICc	AICcWt	ER	LER
1	sex, chronotype, late arrivals, dismissals from class, sick leaves (d)	20	118909.8	0.86	3.01 e-1	1.54	0.19
2	sex, social jetlag, late arrivals, dismissals from class, sick leaves (d)	20	118911.8	2.86	1.11 e-1	4.17	0.62
3	sex, sleep duration on school days, late arrivals, dismissals from class, sick leaves (d)	20	122818.4	3909.36	0	n.a.	n.a.
4	**sex, age, chronotype, late arrivals, dismissals from class, sick leaves (d)**	**21**	**118909**	**0**	**4.61 e-1**	**1**	**0**
5	sex, age, social jetlag, late arrivals, dismissals from class, sick leaves (d)	21	118911.6	2.57	1.27 e-1	3.62	0.56
6	sex, age, sleep duration on school days, late arrivals, dismissals from class, sick leaves (d)	21	122816.9	3907.89	0	n.a.	n.a.
7	sex, age, chronotype	18	118959.3	50.29	5.50 e-12	83,895,618,181.82	10.92
8	sex, age, social jetlag	18	118964.2	55.25	5.00 e-13	922,851,800,000.00	11.96
9	sex, age, sleep duration on school days	18	122880.9	3971.90	0	n.a.	n.a.

The number of estimated parameters (K) for each model is reported. The most parsimonious model for the given data was model 4 (lowest AICc score = 118909). The differences in AICc scores (delta AICc) are calculated for each model relative to model 4. The weight of each model (AICcWt) indicates the weight of evidence for that model to be the best fit for the given data. Model 4 received 46% of the total weight of the models considered. The evidence ratio (ER) is the ratio between the weight of the best model and the weight of each single model. The log evidence ratio (LER) can be used to compare the models. We used Kass and Raftery[57] guidelines to compare the models: LERs greater than 0, 0.5, 1, and 2 indicate respectively "minimal", "substantial", "strong", and "decisive" evidence for model 4 to be the most parsimonious relative to the other models. There was minimal evidence for model 4 to be a better fit of the data compared to model 1. Model 4 had the same set of predictors as model 1, with age as additional predictor. Since age was not significantly related to grades ($b = 0.084$, $t(406) = 1.802$, $p = 0.0723$), we described the effect on grades of the set of predictors in model 1. There was substantial evidence for model 4 to be a better fit of the data compared to model 2 and 5 that had social jetlag among the set of predictors. Social jetlag was not significantly related to grades (model 2: $b = -0.051$, $t(407) = -1.835$, $p = 0.0672$; model 5: $b = -0.055$, $t(406) = -1.960$, $p = 0.0507$). There was decisive evidence for both model 4 and 1 (models with chronotype among the predictors) to be a better fit of the data compared to model 3 and 6 that had sleep duration among the predictors, and compared to models 7, 8, and 9 that did not have the school attendance variables among the predictors.

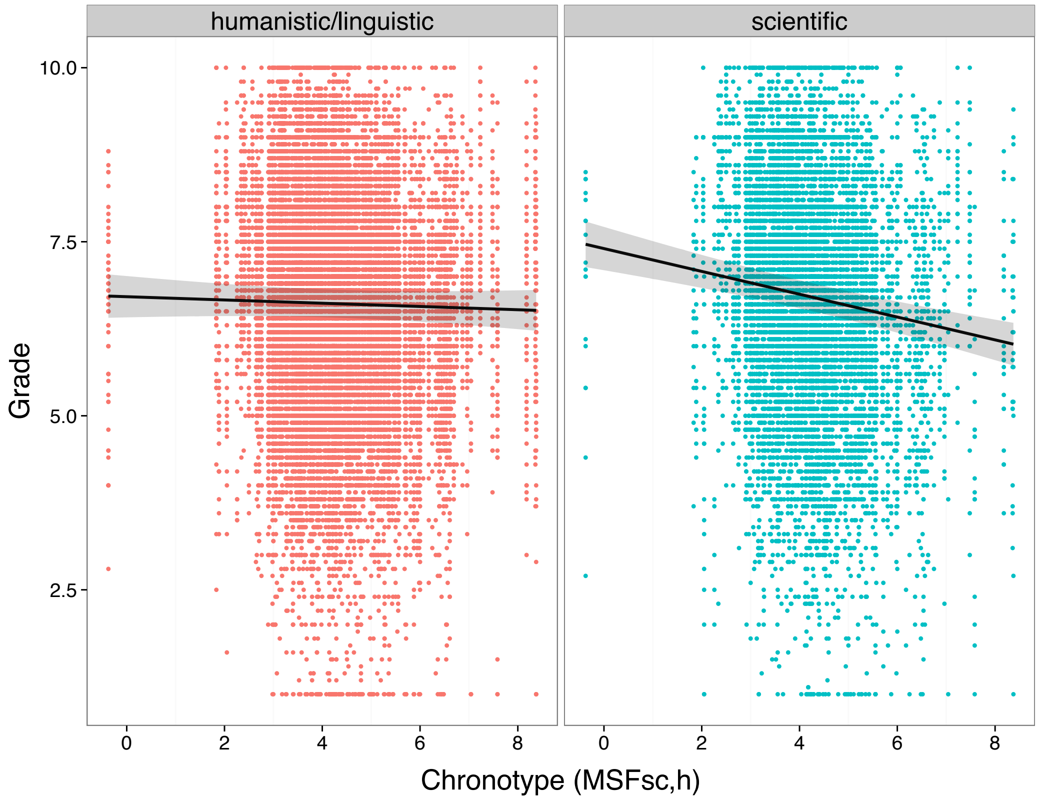


Supplementary Figure S1. Sleep characteristics of all students and of the 20% earliest and 20% latest chronotypes (data from the MCTQ).

Data points represent averages of sleep onset (filled circle), midpoint of sleep (filled triangle), and sleep end (open circle) on school days and on school-free days. Error bars represent standard deviations.



Supplementary Figure S2. Influence of chronotype (MSF_{sc}) on grades by subject.
Data points represent individual grades. The fits of the model with confidence interval (grey area) are plotted. The influence of chronotype on grades was significant only for geography, biology, chemistry, and mathematics.



Supplementary Figure S3. Influence of chronotype (MSF_{sc}) on grades by subject area.
Data points represent individual grades. The fits of the model with confidence interval (grey area) are plotted. The influence of chronotype on grades was significantly stronger for scientific subjects compared with humanistic/linguistic subjects.

Chapter 4

The role of chronotype, time of day, attendance, and study effort in academic performance of university students

Giulia Zerbini, Martha Merrow, and Thomas Kantermann

Abstract

Individuals have different sleep times and diurnal preferences. This can be easily quantified and described as a distribution of chronotypes, ranging from very early (larks) to very late (owls). Several studies have found that chronotype and time of day influence performance. For instance, students with a late chronotype obtain on average worse grades, and this effect is more pronounced when examinations are taken in the early morning. Here we aimed to further explore the relationship between chronotype, time of day, and grades in university students. Grades from examinations taken at 9:00 h, 14:00 h, and 18:30 h were collected. In addition, students reported their class attendance rate, study effort, and stimulants consumption. The interaction effect between chronotype and time of day on grades was not significant. Overall, attendance, study effort, and cigarette consumption were the strongest predictors for academic performance. Increased attendance and study effort were associated with better grades, whereas a higher number of cigarettes smoked (per month) was related to worse grades. A late chronotype was associated with decreased attendance, and increased alcohol consumption, as predicted by earlier studies. The number of grades collected at the three times of day was not evenly distributed, and therefore more research is needed to establish how the chronotype-effect on grades varies with time of day in university students. Results from additional studies would be useful for developing policies to optimize lecture and examinations schedules taking chronotype into account.

Introduction

Performance at every level (from cognitive to physical) is sensitive to the influence of sleep and time of day (Alhola & Polo-Kantola, 2007; Blatter & Cajochen, 2007; Lim & Dinges, 2010; Souissi, Sesboüé, Gauthier, Larue, & Davenne, 2003). For instance, cognitive performance is impaired after sleep deprivation and when certain tasks are performed at night (Dijk, Duffy, & Czeisler, 1992; Lo et al., 2012). Further, individuals differ in terms of sleep timing and diurnal preferences (chronotype; Horne & Ostberg, 1976; Roenneberg, Wirz-Justice, & Merrow, 2003). Sleep timing can be easily measured via the Munich ChronoType Questionnaire (MCTQ; Roenneberg et al., 2003). From the answers to the MCTQ, chronotype can be assessed as the midpoint of sleep on work-free days (MSF), corrected for sleep debt accumulated on workdays (MSF_{sc}). The distribution of chronotypes is wide, ranging from early (morning) to late (evening) types (Roenneberg et al., 2007). The influence of chronotype on performance has been extensively studied in high-school students. Although chronotype is later during adolescence, schools generally start early in the morning, leading to a deficit in performance by late chronotypes when compared to early ones (Crowley et al., 2014; Roenneberg et al., 2004; Tonetti, Natale, & Randler, 2015). Many studies have shown that, on average, late chronotypes obtain lower grades compared to early chronotypes (Borisenkov, Perminova, & Kosova, 2010; Díaz-Morales & Escribano, 2013; Kolomeichuk, Randler, & Shabalina, 2016; Preckel et al., 2013; Rahafar, Maghsudloo, Farhangnia, Vollmer, & Randler, 2016; Randler & Frech, 2006; 2009; van der Vinne et al., 2015; Vollmer, Pötsch, & Randler, 2013). In addition, we have recently shown that the chronotype-effect on grades depends on time of day of testing, with late chronotypes underperforming in the morning, but not in the early afternoon (12:00 h – 15:00 h) (van der Vinne et al., 2015). Similarly other studies have found that late chronotypes are particularly impaired when tested in the morning while their performance improves later in the day (Goldstein, Hahn, Hasher, Wiprzycka, & Zelazo, 2007; Lara, Madrid, & Correa, 2014). This phenomenon is known in literature as the synchrony effect (May, Hasher, & Stoltzfus, 1993). Based on this, we hypothesized that students with a late chronotype would obtain better grades than early chronotypes if tested later in the day. This hypothesis is difficult to test in high-school students because usually examinations are scheduled in the first half of the day. A few studies have shown that late chronotypes attending school in the afternoon obtain comparable grades relative to early chronotypes (Itzek-Greulich, Randler, & Vollmer, 2016; Martin, Gaudreault, Perron, & Laberge, 2016). How would the grades of early and late chronotypes change if students were tested even later in the day (evening) is still unknown. Universities have more flexible schedules, and sometimes examinations can be scheduled early in the morning as well as late in the evening, giving us the unique opportunity to expand our knowledge about the interaction effect between chronotype and time of day on grades.

The aim of this study was therefore to investigate how the effect of chronotype on grades is modulated by time of day in university students that take examinations at very different times of day (from 9:00 h until 18:30 h). Based on previous studies, we expected better grades achieved by early chronotypes in the morning; no difference in grades between early and late chronotypes in the afternoon; and late chronotypes obtaining better grades than early chronotypes in the evening. Since many other factors in addition to chronotype and time of day influence grades, we also assessed attendance rate, study effort, and stimulants consumption of the students.

Methods

The study was conducted at the University of Groningen (53° 13' N/6° 33' E), The Netherlands, in February 2016. Students enrolled in the first and second year at the Departments of Biology and Life and Science Technology (Faculty of Science and Engineering) participated in the study. Students were asked to fill in three questionnaires: the Munich ChronoType Questionnaire (MCTQ; Roenneberg et al., 2003), a questionnaire about stimulants consumption, and a questionnaire about lecture attendance and study effort (see Supplementary Information). The MCTQ asks about sleep timing separately on workdays and on work-free days and is used to assess chronotype as the midpoint of sleep on work-free days (MSF), corrected for sleep debt accumulated on workdays (MSF_{sc}). Most of the students (69%) did not have a regular working schedule, and it was therefore not possible to apply the sleep correction (based on number of workdays) to calculate chronotype. For this reason, we chose to use MSF (not sleep corrected) as a proxy for chronotype. We excluded from the analyses the students who used an alarm clock on work-free days (exclusion criterion used for MSF_{sc} as well). The MCTQ allows also the assessment of other sleep-related variables such as sleep duration on lecture/workdays and social jetlag (the mismatch between the circadian and the social clocks; Wittmann, Dinich, Merrow, & Roenneberg, 2006).
In the stimulants questionnaire, students reported their daily/weekly/monthly consumption of alcohol (beer, wine, strong alcohol), coffee, tea, caffeinated drinks, and sleeping pills/aids. The total amount of alcoholic drinks, cigarettes and cups of coffee consumed per month were analyzed. The attendance and study effort questionnaire asked about lecture attendance (%) and about how much effort (on a scale from 1 to 10) the students put in their studying in order to pass an examination.

Grades from examinations taken between January 2015 and January 2016 were retrieved from the online database of the Faculty of Science and Engineering. In the Dutch university system, grades span from 1 (very bad) to 10 (outstanding), with 6 being the minimum grade to pass an examination (International Recognition Department of Nuffic Netherlands Organisation for International Cooperation in Higher Education, 2013). Date and time of day of each examination were also collected. Examinations were taken at three different times of day: morning (9:00 h), afternoon (14:00 h), and evening (18:30 h). Grades were obtained from examinations of 61 different courses, grouped into 13 subject areas: chemistry, ecology, ethics in research, genetics, human biology, imaging techniques, informatics, mathematics, molecular biology, neurobiology, pharmacology, physics, and physiology.

Statistical analyses were done using R software (R version 3.3.0; The R Core team, 2013). Model selection based on the Akaike's Information Criterion (AIC; Akaike, 1973) was run to select the most parsimonious model for the given data. Grades were analyzed as dependent variable. Student ID was entered in the model as random factor. Different combinations of the following explanatory variables were entered in the models: sex, age, attendance, study effort, number of cigarettes per month, number of drinks (beer, wine, strong alcohol) per month, number of cups of coffee per month, chronotype (MSF), social jetlag (absolute MSF-MSW), and sleep duration on work/lecture days. Subject area, month, day of the week, time of day were analyzed as covariates. The estimates of the model are indicated in the results as "b" coefficients. To compare the strength of the effects of the different predictors the coefficients were standardized and are indicated as "β" coefficients.

In addition, we ran a separate linear mixed model with grades as dependent variable and chronotype, time of day, and the interaction between chronotype and time of day as explanatory variables. Chronotype and time of day were analyzed as categorical variables. Students were divided into two equal-sized groups of early (MSF ≤ 5.50) and late (MSF > 5.50) chronotypes. The variable time of day had three levels: morning (9:00 h), afternoon (14:00 h), and evening (18:30 h). Based on the model selection previously described, student ID was analyzed as random factor, and subject area and day of the week when the examinations had been taken were analyzed as covariates.

To investigate the influence of sex, age, and sleep-related variables on attendance, effort and stimulants consumption different regression analyses were run based on the distribution of the dependent variables. To account for negative skewedness in the attendance data, we ran a quantile regression aiming to estimate the conditional median. The effort data were normally distributed allowing the use of linear regression. To account for excessive zeros and over dispersion of the stimulants data we used a zero-inflation model with negative binomial distribution. Model selection based on the AIC was again applied.

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 Centre of Groningen (NL) approved the study. All participants signed an informed consent.

Results

A total of 258 students (141 females and 117 males, mean age 20.11 ± 1.50 SD; age range 18-27 years) participated in the study. Of these students, 36 reported using an alarm clock on work-free days and were therefore excluded from all analyses. The final sample size was of 222 students (121 females and 101 males, mean age 20.15 ± 1.52 SD, age range 18-27 years). 1241 grades (average number of grades per student: 5.59) from examinations taken between January 2015 and January 2016 were collected. 548 grades were collected from examinations taken in the morning (9:00 h), 620 grades from examinations taken in the afternoon (14:00 h) and 73 grades from examinations taken in the evening (18:30 h). The students filled in three questionnaires: the MCTQ, a questionnaire about stimulants consumption, and a questionnaire about lecture attendance and study effort. The descriptive statistics of the data collected with these questionnaires are summarized in Table 1.

Chronotype, time of day, and grades

First we ran a mixed model with chronotype and time of day as independent variables to test the hypothesis that grades in university students depend on the interaction between chronotype and time of day of testing. Subject area and day of the week were analyzed as covariates. We did not find any significant interaction effect between chronotype and time of day on grades ($F_{2,998} = 2.622$, $p = 0.0732$). Overall, late chronotypes obtained lower grades (main effect of chronotype: $F_{1,220} = 6.377$, $p = 0.0123$), but the chronotype-effect on grades was not modulated by time of day.

Table 1. Demographics of 121 female and 101 male first- and second-year university students (data from the Munich ChronoType Questionnaire).

Outcome measure	Average (± SD)	Range
Age (years)	20.15 (1.52)	18 - 27
Chronotype (MSF, h)	5.71 (1.12)	2.63 - 11
Social Jetlag (h)	1.68 (0.83)	0 - 5.38
Sleep onset on work/lecture days (h)	0.22 (0.95)	-2.75 - 3.08
Sleep end on work/lecture days (h)	7.83 (0.99)	5.75 - 12
Sleep duration on work/lecture days (h)	7.61 (1.05)	5 - 10.97
Sleep onset on work-free days (h)	1.32 (1.26)	-1.75 - 6
Sleep end on work-free days (h)	10.09 (1.24)	7 - 16
Sleep duration on work-free days (h)	8.77 (1.11)	5.17 - 12.5
Attendance (scale 0-100)	88 (15.06)	2 - 100
Effort (scale 0-10)	6.88 (1.28)	3 - 10
Alcohol (number drinks per month)	33.30 (36.25)	0 - 168
Coffee (cups per month)	27.79 (36.36)	0-180
Cigarettes (per month)	6.57 (36.53)	0 - 360
Grades	6.72 (1.40)	0.5-10

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). Data concerning lecture attendance, study effort, stimulants consumption, and grades are also reported.

However, only 5% of the grades were collected in the evening (45% in the morning and 50% in the afternoon), decreasing the predictive power of the model for the evening grades (higher standard deviation; SD morning: 1.26; SD afternoon: 1.44, SD evening: 1.93). Therefore, we decided to repeat the same analysis and exclude the evening examinations. When only the examinations taken in the morning and in the afternoon were considered, the interaction effect between chronotype and time of day was significant ($F_{1,927}$ = 5.740, p = 0.0168). Post hoc tests (with Bonferroni correction for multiple comparisons) revealed that early chronotypes obtained higher grades (almost half grade higher) in the afternoon compared to late chronotypes, while this difference was not significant in the morning (afternoon: b = 0.4, t (329.5) = 2.47, p = 0.02; morning: b = 0.2, t (410.4) = 1.48, p = 0.28, Fig. 1).

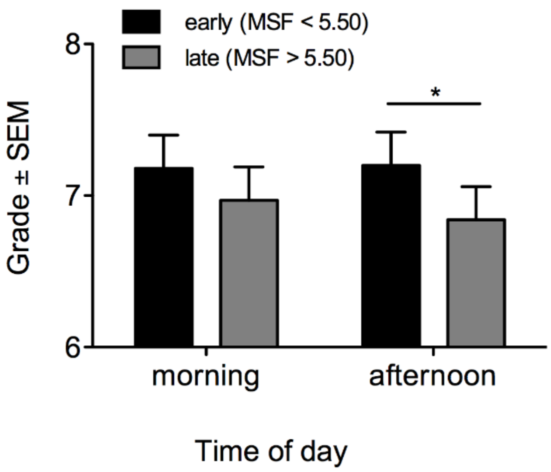


Figure 1. Interaction effect between chronotype and time of day of examinations on grades.
Bars represent means with standard error of the mean (SEM) of grades from examinations taken at two times of day: morning (9:00 h) and afternoon (14:00 h). Black bars represent grades from early chronotypes (MSF ≤ 5.50), and grey bars represent grades from late chronotypes (MSF > 5.50). Means and SEM were derived from the multilevel model used to fit the data and therefore represent the estimated means and SEM. The interaction effect between chronotype and time of day was significant: early chronotypes obtained better grades in the afternoon (14:00 h) compared with late chronotypes. *p < .05 (post hoc tests with Bonferroni correction).

The influence of attendance, study effort, and cigarettes consumption on grades

The most parsimonious model to describe the variance in grades had attendance, study effort, and cigarettes consumption among the predictors. Subject area and day of the week were also significantly associated with grades in this model, and were analyzed as covariates. A complete overview of the models considered is reported in Table S1 (Supplementary Information). For both attendance and study effort the correlation with grades was positive (attendance: b = 0.020, t (218) = 5.112, p < .0001; study effort: b = 0.137, t (218) = 2.912, p = 0.0040). The model predicts that if a student increases the attendance rates of 10%, his/her average grade will increase with a factor of 0.2 (on a scale from 1 to 10). The model also predicts that if a student increases the study effort of 1 unit (on a scale from 1 to 10), his/her average grade will increase with a factor of 0.14. The number of cigarettes consumed per month negatively correlated with grades (b = -0.004, t (218) = -2.377, p = 0.0183). For each additional cigarette consumed per month, the model predicts a decrease in grades with a factor of 0.004. Attendance showed the strongest association with grades (β = 0.223), followed by study effort (β = 0.124), and cigarettes consumption (β = -0.093).

The influence of sex, age, chronotype, and attendance on study effort

The stepwise backward linear regression analysis revealed that the most parsimonious model to describe the variance in study effort had sex, age, chronotype, and attendance among the predictors. Males had a lower score on this scale compared to females (b = -0.415, t (196) = -2.258, p = 0.0251). In particular, the model predicts that the study effort in a male student is 0.4 points lower than for females on a scale from 1 to 10. Students who were older and attended more often the lectures were characterized by an increased study effort (age: b = 0.111, t (196) = 1.990, p = 0.0480; attendance: b = 0.012, t (196) = 2.087, p = 0.0382). Finally, chronotype was negatively associated with study effort (b = -0.257, t (196) = -3.014, p = 0.0029, Fig. 2A). The model predicts that a student with 1-hour later chronotype shows a decrease in study effort of a factor of 0.3 (on a scale from 1 to 10). The effect on study effort was strongest for chronotype (β = -0.216), followed by sex (β = -0.160), age (β = 0.130), and attendance (β = 0.144).

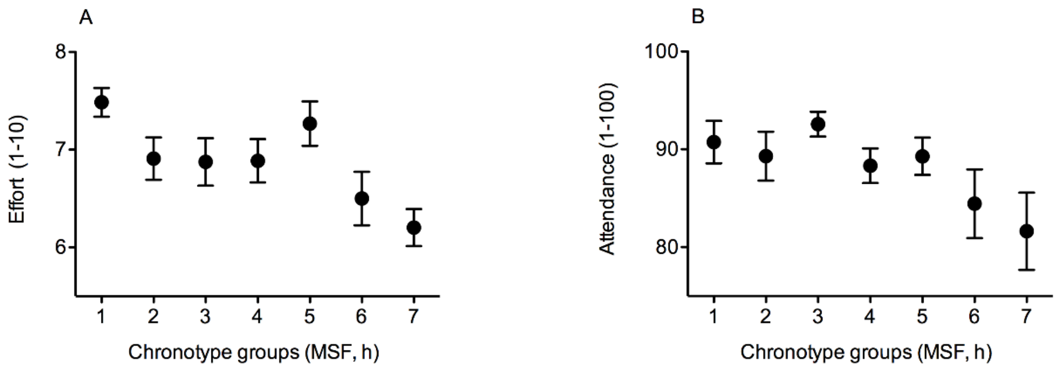


Figure 2. Influence of chronotype on study effort and attendance rates.
Data points represent mean study effort scores (A) and attendance rates (B) with standard error of the mean (SEM) of 7 equal-sized chronotype groups, with 1 being the earliest and 7 the latest chronotype group. The ranges of chronotype for each group are: (1) 2.63-4.66, (2) 4.67-5.08, (3) 5.09-5.38, (4) 5.39-5.64, (5) 5.65-6.21, (6) 6.22-6.85, and (7) 6.86-11. Late chronotypes were more likely to put less effort in studying and to attend fewer lectures.

The influence of chronotype, social jetlag, sleep duration, and stimulants consumption on attendance

The stepwise backward quantile regression analysis revealed that the most parsimonious model to describe the variance in attendance had chronotype, social jetlag, sleep duration on lecture/workdays, alcohol and cigarettes consumption among the predictors. Although cigarettes consumption was not significantly associated with attendance (b = -0.44, t (216) = -

1.786, p = 0.0755), removing this predictor from the model increased the AIC from 1529.6 to 1533.8 (worse fit). Chronotype, sleep duration on lecture/workdays, and alcohol consumption were negatively associated with attendance (chronotype: b = -2.413, t (216) = -2.693, p = 0.0076; sleep duration: b = -1.502, t (216) = -2-080, p = 0.0279; alcohol consumption: b = -0.094, t (216) = -2.544, p = 0.0117). Social jetlag was positively correlated to attendance (b = 2.934, t (216) = 2.213, p = 0.0279). The model predicts that a student with 1-hour later chronotype has a 2% decrease in attendance (Fig. 2B).

The influence of sex and chronotype on stimulants consumption

The model with sex and chronotype as predictors was the most parsimonious model to describe the variance in alcohol consumption. Chronotype influenced the likelihood of consuming alcohol (b = -0.804, z = -2.638, p = 0.0083; Fig. 3). A student with a 1-hour earlier chronotype had 2 times larger odds of never consuming alcohol. Chronotype and sex were both positively associated with the amount of alcohol consumed, with late chronotypes and male students consuming significantly more alcohol (chronotype: b = 0.258, z = 3.587, p = 0.0003; sex: b = 0.456, z = 3.250, p = 0.0012).

Finally, none of the variables of interest was found to be significantly associated with cigarettes consumption and coffee intake.

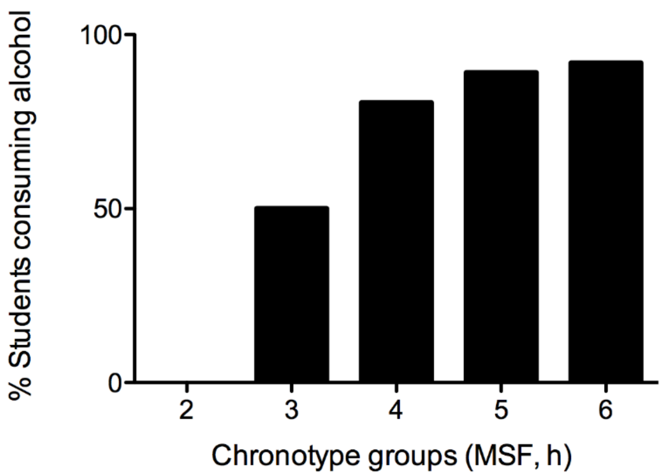


Figure 3. Influence of chronotype on alcohol consumption.
The percentages of students drinking at least one alcoholic drink per month were calculated in relation to the entire sample and per hourly bin based on the students' MSF. Among the students with a late chronotype there is an increased number of students drinking at least one alcoholic drink per month. The number of students per each hourly MSF bin was: 1 (MSF = 2), 8 (MSF = 3), 41 (MSF = 4), 92 (MSF = 5), 49 (MSF = 6).

Discussion

The influence of chronotype on academic performance has been shown both in high school and university students. In general, late chronotypes report lower achievements compared to early chronotypes (Beşoluk, Önder, & Deveci, 2011; Borisenkov et al., 2010; Preckel, Lipnevich, Schneider, & Roberts, 2011; Tonetti et al., 2015). Several studies have suggested that this difference in performance might be related to the early school/university starting times that handicap students with a late chronotype (Preckel et al., 2013; Randler & Frech, 2006; 2009; van der Vinne et al., 2015; Vollmer et al., 2013). The importance of timing of examination has been shown in recent studies where late chronotypes obtained lower grades in the morning, while no difference in performance between early and late chronotypes was found in the afternoon (Itzek-Greulich et al., 2016; Martin et al., 2016; van der Vinne et al., 2015).

Here, we aimed to better elucidate how the chronotype-effect on grades changes with time of day by collecting grades from examinations taken in the morning, afternoon, and evening (9:00 h, 14:00 h, and 18:30 h). The interaction effect between chronotype and time of day of testing was not significant. However, the number of grades collected at the three times of day was not evenly distributed, with fewer grades (5%) collected during evening examinations compared to morning (45%) and afternoon (50%) examinations. When we considered only the examinations taken in the morning and in the afternoon, we found that early chronotypes obtained better grades in the afternoon, whereas there was no significant difference between early and late chronotypes in the morning. This finding did not support our hypothesis that late chronotypes would obtain worse grades in the morning and better grades later in the day compared to early chronotypes. However, we based our hypothesis on previous studies done in high-school students, but university and high-school students differ in many aspects. For instance, the chronotype of university students is on average later than high-school students: chronotype starts delaying during adolescence and reaches the peak in lateness when young adults are around 20 years old (mean age in our sample was 20.15 years; Roenneberg et al., 2004). For this reason, the morning examination session might have been too early even for early chronotypes, with the afternoon session being their optimal time for taking an examination. In addition, the more flexible schedules typical of university might have reduced the overall chronotype and time of day influence on grades. This idea is supported by the findings of a recent meta-analysis where the chronotype-effect on grades was found to be stronger in high-school students, that usually have regular schedules starting early in the morning, compared with university/college students (Tonetti et al., 2015).

Further, our results suggest that attendance rate and study effort could be more important variables for academic success than chronotype in university students. The most parsimonious model to predict grades had attendance rate and study effort, but not chronotype, among the significant predictors. Chronotype was significantly associated with grades, only when the other predictors, such as attendance and study effort, were not added to the model. In line with these results, previous studies have found that other variables, such as stimulants consumption and class attendance, are more strongly associated with academic achievements

(Gomes, Tavares, & de Azevedo, 2011; Onyper, Thacher, Gilbert, & Gradess, 2012). This suggests that a complex interaction of many factors influence school and academic performance, requiring future studies to systematically assess all these variables, trying to discern the strength of their unique contribution in determining grades. For instance, similarly to our previous results (chapter 3), we found that chronotype was significantly associated with attendance, with late chronotypes being more likely to attend fewer lectures than early chronotypes. In the same study, absenteeism was found to be negatively associated with grades. Together, these data suggest that early schedules (both at the school and university level) challenge students with a late chronotype, influencing negatively their attendance and possibly their grades.

In line with previous studies, we found that chronotype was related to stimulants consumption, with late chronotypes consuming more alcohol (Adan, 1994; Randler, 2008; Wittmann et al., 2006; Wittmann, Paulus, & Roenneberg, 2010). We did not find any significant influence of stimulants consumption on grades. However, Onyper and colleagues found that alcohol consumption was the strongest among different predictors of academic achievements in university students (Onyper et al., 2012). The relationship between chronotype, stimulants consumption, and school/academic performance needs therefore further elucidation.

A limitation of this study is the correlational approach, not making possible any causal inference. In addition, we used questionnaires (to assess attendance rate and study effort) that had not been previously validated. Finally, we were not able to definitively confirm or reject our hypothesis of an interaction effect between chronotype and time of day on grades because of the uneven number of exams taken at the three different times of day considered.

Taken together, although we did not find a significant association between chronotype, time of day, and grades in university students, chronotype was still found to be associated with reduced attendance and increased stimulants consumption, which in turn have been found to be negatively related to grades in our and previous studies (chapter 3; Gomes et al., 2011; Onyper et al., 2012; Roby, 2004). More research is needed in university students to clarify the role of chronotype and other variables such as stimulants consumption and class attendance in relation to academic performance. Moreover, future studies should assess in more detail the academic performance of early and late chronotypes at different times of day, considering also the lecture schedules that could influence a student's ability to learn and prepare for the examination.

Acknowledgments

We thank Eline Struik and Marieke Harms for their help with the data collection. Our work is supported by the Technology foundation STW grant P10-18/12186 and the University of Groningen.

Supplementary Information

Questionnaire about stimulants consumption, lecture attendance, and study effort.

Deelnemersenquête

Beste deelnemer, wees ervan verzekerd dat alle informatie uit deze enquête anoniem geanalyseerd wordt. Alle data is aan strikte geheimhouding onderworpen, en zal niet aan derden doorgegeven worden.

Datum ____________
Studentnummer____________

Consumptie (a.u.b. gemiddelde waarden aangeven!)	**per → dag**	**/ week**	**/ maand**
a) Ik rook _____ sigaretten ...	☐	☐	☐
b) Ik drink _____ glazen bier ...	☐	☐	☐
c) Ik drink _____ glazen wijn …	☐	☐	☐
d) Ik drink _____ glazen sterke drank (jenever/whisky/wodka/etc.) …	☐	☐	☐
e) Ik drink _____ koppen koffie …	☐	☐	☐
f) Ik drink _____ koppen zwarte thee ...	☐	☐	☐
g) Ik drink _____ blikjes cafeïne houdende frisdrank …	☐	☐	☐
h) Ik neem slaap bevorderende medicamenten _____ maal in ...	☐	☐	☐

Motivatie
a) Ik ben gemiddeld aanwezig bij _____% (percentage aangeven) van mijn colleges/werkcolleges/practica.
b) Ik besteed gemiddeld op een schaal van 1-10 _____ tijd en moeite om een vak met een voldoende cijfer af te sluiten.

Bedankt voor het invullen van onze enquête!

Table S1. Description of the 10 models used to explore the influence of the explanatory variables (chronotype, sleep duration on workdays, social jetlag, attendance rate, study effort, and stimulants consumption) on grades.

Model	Explanatory variables	K	AICc	Delta AICc	AICcWt	ER	LER
1	Attendance rate, study effort, chronotype (MSF)	22	4045.9	5.1	3.7 e-2	12.9	1.1
2	Cigarettes, alcohol, and coffee consumption (monthly), chronotype (MSF)	23	4046.2	5.4	3.1 e-2	15.1	1.2
3	Attendance rate, study effort, alcohol consumption (monthly), chronotype (MSF)	23	4047.4	6.7	1.7 e-2	28.0	1.4
4	Attendance rate, study effort, social jetlag (SJL)	22	4045.8	5.0	3.9 e-2	12.2	1.1
5	Attendance rate, study effort	21	4044.4	3.7	7.6 e-2	6.2	0.8
6	**Attendance rate, study effort, cigarettes consumption (monthly)**	**22**	**4040.8**	**0**	**4.8 e-1**	**1**	**0**
7	Attendance rate, study effort, sleep duration workdays (SDw)	22	4045.0	4.2	5.7 e-2	8.3	0.9
8	Attendance rate, study effort, cigarettes consumption (monthly), chronotype (MSF)	23	4042.6	1.8	1.9 e-1	2.5	0.4
9	Attendance rate, study effort, cigarettes and alcohol consumption (monthly), chronotype (MSF)	24	4044.4	3.7	7.6 e-2	6.2	0.8
10	Chronotype (MSF)	20	4079.1	38.4	2.2 e-9	214,298,059.6	8.3

The number of estimated parameters (K) for each model is indicated. The most parsimonious model for the given data was model 6 (lowest AICc score = 4040.8). The differences in AICc scores (delta AICc) are calculated for each model relative to model 6. AICcWt indicates the weight of evidence for that model to be the best fit for the given data. Model 6 received 48% of the total weight of the models considered. The evidence ratio (ER) is the ratio between the weight of the best model (model 6) and the weight of each single model. The log evidence ratio (LER) can be used to compare the models. We used the following guidelines to compare the models: LERs greater than 0, 0.5, 1, and 2 indicate respectively "minimal", "substantial", "strong", and "decisive" evidence for model 6 to be the most parsimonious relative to the other models (Kass & Raftery, 1995). There was minimal evidence for model 6 to be a better fit of the data compared to model 8. The two models had the same sets of predictors, except for chronotype (MSF) that was only present in model 8. However, chronotype was not significantly associated with grades ($b = -0.028$, $t(217) = -0.504$, $p = 0.6149$) in model 8, and therefore we decided to report the results of model 6. There was substantial evidence for model 6 to be a better fit of the data compared to models 5, 7, and 9. There was strong evidence for model 6 to be a better fit of the data compared to models 1,2, 3, and 4. Finally there was decisive evidence for model 6 to be a better fit of the data compared to model 10.

Chapter 5

Time to learn: How chronotype impacts education

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Abstract

A growing body of literature has linked chronotype and sleep to school performance. Chronotype is under the control of the circadian clock and refers to sleep timing and diurnal preferences. Chronotype changes with age and is latest during adolescence, giving rise to a mismatch between the (late) circadian clock and the (early) school clock. In general, evening (late) chronotypes obtain lower grades. School performance is influenced by many other factors such as motivation, intelligence, and conscientiousness. Some of these factors also relate to chronotype. The present paper reviews the literature on the relationship between chronotype and school performance, with the aim of suggesting hypotheses about the mechanisms behind this complex phenomenon and exploring solutions for an optimized school system. Based on the literature reviewed, we hypothesize that chronotype has both a direct and an indirect effect on school performance. The indirect effect is mediated by factors such as conscientiousness, achieving and learning motivation, mood, and alertness. In addition, time of day of testing plays an important role since the chronotype-effect on grades is strongest in the morning and disappears in the afternoon. Strategies to decrease the mismatch between the adolescent circadian clock and the school clock could involve light interventions to advance the students' sleep timing, delays in school starting times, and rearrangements of tests schedules (tests later in the day).

Introduction

Education plays an important role in determining future career opportunities (French, Homer, Popovici, & Robins, 2015; Ma, Pender, & Welch, 2016). School achievements (e.g. grades) are often used as selection criterion for admission to universities and are also among the best predictors for academic success after high school (Geiser & Santelices, 2007). Many factors influence school achievements. Intelligence, academic motivation, and conscientiousness are examples of individual characteristics relevant for obtaining good grades (Bratko, Chamorro-Premuzic, & Saks, 2006; Deary, Strand, Smith, & Fernandes, 2007; Fortier, Vallerand, & Guay, 1995). In addition, many other factors not under the direct control of students, such as socio-economic status, parental involvement and quality of the teachers, influence how they perform in school (Juang & Silbereisen, 2002; Pokropek, Borgonovi, & Jakubowski, 2015; Rockoff, 2004). Sleep and chronotype (a function of the circadian clock) are examples of biological factors that contribute to success in school. In this review, we discuss the influence of chronotype on school achievements, in order to develop hypotheses concerning the mechanisms behind this phenomenon.

What is chronotype and how is it measured?

The term "chronotype" has been used in literature in relation to both sleep timing (Roenneberg, Wirz-Justice, & Merrow, 2003) and to the psychological construct 'diurnal preference' (Horne & Ostberg, 1976). In both cases, chronotype is assessed with simple questionnaires (discussed below). Depending on the questionnaire used, chronotype is expressed as a continuous variable (e.g. the Munich Chronotype Questionnaire, MCTQ; Roenneberg et al., 2003) or as a categorical variable (e.g. the Morningness-Eveningness Questionnaire, MEQ; Horne & Ostberg, 1976). Although chronotype can be assessed and expressed in different ways, there is consensus that chronotype is under the control of the circadian clock. The circadian clock confers rhythmicity on many biological processes, from molecules to behaviors, and uses light as main zeitgeber (time cue) to entrain (synchronize) behavior to the external light-dark cycle (Roenneberg & Merrow, 2007; Roenneberg, Kumar, & Merrow, 2007). The variability in the light landscape, as well as in the biological and genetic background of individual circadian clocks, leads to a wide distribution of phases of entrainment to the external light-dark cycle. Chronotype (at least using the MCTQ) is an estimate of an individual phase of entrainment via subjective assessment of sleep timing.

In this section, we explain how sleep timing is regulated, how sleep and chronotype can be measured with different questionnaires, and that chronotype is later during adolescence, a particularly relevant finding with respect to school performance.

The regulation of sleep

According to the two-process model, the timing and consolidation of sleep is regulated by a circadian and a homeostatic process (Borbély, 1982; Daan, Beersma, & Borbély, 1984). Sleep pressure (homeostatic process) increases during wakefulness and is dissipated during sleep.

The circadian clock promotes wakefulness during the day and sleep during the night (in diurnal animals). The likelihood of falling asleep increases when sleep pressure is high and when the circadian clock stops promoting wakefulness and starts promoting sleep. The two-process model predicts various sleep characteristics of properties that are interesting for society and behavior. For instance, the model predicts that sleep duration is shorter when sleep occurs outside the circadian window for sleep, thus predestining shift workers – or those that need an alarm clock to match biological and social clocks - to regular sleep deprivation. Experimental data collected before and after the model was proposed support this observation and other predictions, such as REM sleep being highest when the end of the sleep episode coincides with the end of the circadian sleep window (Akerstedt & Gillberg, 1981; Czeisler, Weitzman, & Moore-Ede, 1980; Dijk & Czeisler, 1995). The two-process model has been amended by various researchers over the years to reflect real life observations, for instance age-related changes in sleep (Borbély & Achermann, 1999; Phillips, Chen, & Robinson, 2010; Skeldon, Derks, & Dijk, 2016).

Estimating chronotype with questionnaires

The timing of sleep is thus viewed as a circadian clock-regulated trait. Sleep timing is subjectively assessed via the Munich ChronoType Questionnaire (MCTQ; Roenneberg et al., 2003). This short questionnaire asks about behavior separately on school (or work) days and on school-free (or work-free) days. Chronotype is calculated from the MCTQ as the midpoint of sleep on school-/work-free days (MSF), corrected for sleep debt accumulated on school/workdays (MSF_{sc}). Chronotype is expressed as local clock time and ranges from early (early midpoint of sleep) to late (late midpoint of sleep) chronotypes, with most individuals hovering in the middle, forming almost a normal distribution. Unlike the MCTQ, which derives chronotype from sleep timing on school/work-free days, the MEQ assesses chronotype based on diurnal preferences (e.g. preferred time of day to perform physical and mental work; Horne & Ostberg, 1976). With this questionnaire, chronotype is categorized as a score (range: 16-86), with high numbers corresponding to morning types (59 and above), low numbers corresponding to evening types (41 and below) and numbers between 42 and 58 corresponding to intermediate types. In 1993, an adapted version of the MEQ was developed for children and adolescents: the Morningness-Eveningness Scale for Children (Carskadon, Vieira, & Acebo, 1993). There are also other questionnaires assessing chronotype, such as the Composite Scale of Morningness (reviewed in Levandovski, Sasso, & Hidalgo, 2013).

MCTQ and MEQ answers are generally highly correlated ($r = -0.73$; Zavada, Gordijn, & Beersma, 2005). We prefer to use the MCTQ, as it assigns a putative internal clock time rather than a general score, a more useful feature for e.g. trying to understand the interaction of internal and external time. Concerning validity of the two instruments, chronotype assessed via MCTQ and MEQ also correlates with dim-light melatonin onset (DLMO) (MEQ: $r = -0.70$; MCTQ: $r = 0.68$; Kantermann, Sung, & Burgess, 2015). DLMO is considered a physiological measure of phase of entrainment of the circadian clock (Arendt, 2006; Klerman, Gershengorn, Duffy, & Kronauer, 2002). The levels of the hormone melatonin rise in the evening, are high during the night, and low during the day. This 24-hour rhythm in melatonin production and secretion is under the control of the circadian clock and is stable and robust

against the influence of external factors (except for light, that directly suppresses melatonin). Melatonin concentrations are measured in saliva or plasma using several methods. However, given that these tend to be costly, estimating chronotype or entrained phase with questionnaires permits larger scale studies. The MCTQ online database currently contains well over 200,000 entries.
To the best of our knowledge, there are no studies that have correlated DLMO to school achievements, while many studies have investigated the relationship between chronotype and school performance.

Adolescents have a late chronotype
The decisive years when students are sorted into those that will continue to higher education such as university and those that will cease their formal education generally coincide with adolescence. Several studies have reported that adolescents sleep longer and later than adults (Crowley et al., 2014; Klerman & Dijk, 2008; Roenneberg et al., 2004). The reasons for a delay in sleep timing (and chronotype) in adolescents have already been reviewed elsewhere (Crowley, Acebo, & Carskadon, 2007; Hagenauer, Perryman, Lee, & Carskadon, 2009; Jenni, Achermann, & Carskadon, 2005). A delay in chronotype is observed also in animals (some mammals, such as rhesus monkeys, laboratory rats and mice), suggesting that systematic changes occur during puberty that (delay) the circadian clock as opposed to purely societal structures (Hagenauer et al., 2009).

One explanation for a late chronotype or phase of entrainment in adolescence could lie in (as yet unidentified) hormonal changes regulating downstream physiologies to change chronotype. An alternative explanation comes from an exploration of light sensitivity in adolescence. Time of day-specific or general changes in sensitivity to light could both lead to a change in entrained phase. A rule of thumb is that exposure to light during the end of the subjective (internal) night induces phase advances, while exposure to light during the beginning of the subjective (internal) night induces phase delays (Khalsa, Jewett, Cajochen, & Czeisler, 2003). Therefore, a decreased sensitivity to light in the morning and an increased sensitivity to light in the evening would lead to a delayed phase of entrainment. In a recent study, older (late/post pubertal stage) adolescents were found to be less sensitive to light both in the morning and in the evening when compared to younger (early/mid pubertal stage) adolescents (500 lux morning light, $p = .06$; 500 lux evening light, $p < .05$; Crowley, Cain, Burns, Acebo, & Carskadon, 2015). An overall decrease in sensitivity to light seems therefore related to a delayed phase of entrainment in adolescents, rather than an increase in sensitivity to light in the evening hours. In line with this, the strength of the zeitgeber is also related to phase of entrainment: individuals with less daily light exposure show in general a later phase of entrainment (Roenneberg & Merrow, 2007; Wright et al., 2013). Interestingly, this correlation was not found in adolescents (15 to 20 years old), suggesting again that this age group could be in general less sensitive to light (Roenneberg et al., 2015).
In addition to physiological changes relevant for the circadian timing system, there is evidence that the homeostatic process is altered in adolescents. The build-up of sleep pressure is slower in older adolescents, allowing them to stay awake later (Jenni et al., 2005; Taylor, Jenni, Acebo, & Carskadon, 2005). Exposure to additional evening light (not necessarily an

increased sensitivity) would lead to a later entrained phase (Crowley et al., 2015). The use of electronic media before going to bed, very common in this age group, has been associated with delayed and disturbed sleep (Cain & Gradisar, 2010; Munezawa et al., 2011; van den Bulck, 2004). General difficulties with sleeping and morning tiredness have also been reported in adolescents who consume high doses of caffeine (Orbeta, Overpeck, Ramcharran, Kogan, & Ledsky, 2006).

Psychosocial factors, such as an increased need of independency, could also contribute in influencing sleep timing in adolescents. For instance, when parents dictate bedtimes, they are earlier (Short et al., 2011). This could also lead to earlier entrained phase via restriction of evening/nighttime light exposure.

In conclusion, both modifications in the circadian and in the homeostatic processes, such as altered sensitivity to light and to sleep pressure, may contribute to a delay in sleep timing. In addition, the consumption of caffeine, the use of electronic media in the evening, and the self-selected bedtimes are examples of other factors that can exacerbate this phenomenon.

Chronotype and school performance

The influence of chronotype on school performance has been extensively studied during the past 20 years. Although different chronotype questionnaires have been used, consistent findings about late (evening) types obtaining lower school achievements have been reported (Arbabi, Vollmer, Dörfler, & Randler, 2014; Borisenkov, Perminova, & Kosova, 2010; Díaz-Morales & Escribano, 2013; Giannotti, Cortesi, Sebastiani, & Ottaviano, 2002; Kolomeichuk, Randler, & Shabalina, 2016; Preckel et al., 2013; Rahafar, Maghsudloo, Farhangnia, Vollmer, & Randler, 2016; Randler & Frech, 2006; 2009; Roeser, Schlarb, & Kübler, 2013; Short, Gradisar, Lack, & Wright, 2013; van der Vinne et al., 2015; Vollmer, Pötsch, & Randler, 2013; Warner, Murray, & Meyer, 2008). However, the mechanism(s) behind this phenomenon are still unclear. The influence of chronotype on school performance could take place during the learning (at school and at home) and/or the evaluation phase or both. In addition, the effect of chronotype on school achievements could be direct and/or could be mediated by other factors that are relevant for performance.

Lower school performance in late/evening chronotypes

Several studies have used the MEQ or the adapted versions of the MEQ for children and adolescents (MESC; Carskadon et al., 1993, and PMEQ; Randler & Frech, 2006) to assess the effect of chronotype on official or self-reported grades in high-school students (Díaz-Morales & Escribano, 2013; Escribano, Díaz-Morales, Delgado, & Collado, 2012; Giannotti et al., 2002; Preckel et al., 2013; Randler & Frech, 2009). In all these studies, evening types obtained lower grades compared to morning types. The MEQ score is also correlated with sleep duration, age, and sex: adolescents with a stronger preference for eveningness usually sleep shorter on school nights, are older, and are more likely to be males (Owens, Dearth-Wesley, Lewin, & Gioia, 2016; Tonetti, Fabbri, & Natale, 2008). When controlling for total sleep time, sex, and age, evening types still obtained lower grades (Díaz-Morales & Escribano, 2013; Escribano et al., 2012; Randler & Frech, 2009). Giannotti et al. (2002) performed a regression analysis to assess the impact of 12 independent variables (variables related to substance use, emotional aspects, circadian preference, demographics, and sleep) on school performance. The authors found that evening preference was among the predictors for poor school performance together with emotional problems, substance use, and sex. In another study, eveningness (measured with the Lark-Owl Chronotype Indicator; R. D. Roberts, 1998) was found to be negatively correlated to self-reported final school year grades when controlling for other factors relevant for school achievements such as cognitive ability, conscientiousness, achievement motivation, need for cognition, and daytime sleepiness (Preckel et al., 2013). The influence of circadian preference was also shown on the final high-school exam (often used as admission criterion to Universities), with evening types scoring lower compared to morning types (Randler & Frech, 2006).

Studies that have assessed chronotype using other questionnaires, such as the MCTQ and the CSM (Composite Scale of Morningness; Smith, Reilly, & Midkiff, 1989), found the same chronotype-effect on grades, with late chronotypes obtaining lower grades compared to early chronotypes (Borisenkov et al., 2010; Kolomeichuk et al., 2016; van der Vinne et al., 2015; Vollmer et al., 2013).

Two recent meta-analyses have analyzed the relationship between chronotype and school/academic performance both in high school and university students (Preckel, Lipnevich, Schneider, & Roberts, 2011; Tonetti, Natale, & Randler, 2015). Preckel et al. (2011) performed two separate meta-analyses to explore the relationship between morningness and academic achievements, and eveningness and academic achievements. They found that morningness was positively ($r=0.16$) associated with academic achievements, while eveningness was negatively ($r=-0.14$) associated with academic achievements. Similarly, the meta-analysis of Tonetti, Natale et al. (2015) showed that evening types have worse academic achievements. A separate analysis for studies done in high school and university students revealed that the effect of chronotype was stronger in high-school students.

Taken together, several studies that have assessed chronotype, while controlling for different factors, have found that evening (late) types obtain lower achievements at high school. However, the strength of the relationship between chronotype and school achievements can vary between studies depending on the covariates assessed. For instance, Díaz-Morales and Escribano (2015) reported that the MESC score in their sample of high-school students was related to school achievements only when controlling for age; the significant correlation disappeared when other sleep-related variables, such as time in bed, were added in a multivariate linear regression model. Especially when considering both chronotype and sleep duration, it is difficult to disentangle the effects of these two variables on school performance. Chronotype is tightly linked to sleep duration, with late chronotypes in general sleeping shorter on school/work days (Roenneberg, Kuehnle, et al., 2007). Although the negative effect of short sleep duration on school achievements has been described in many reviews, it is not clear how much of this effect is related to being a late chronotype and how much to sleeping too little (Curcio, Ferrara, & De Gennaro, 2006; Dewald, Meijer, Oort, Kerkhof, & Bögels, 2010; Taras & Potts-Datema, 2005; Wolfson & Carskadon, 2003). Borisenkov et al. (2010) reported a two-fold stronger effect of chronotype on school grades relative to sleep duration, suggesting that chronotype should be included in future research about the effect of sleep on school performance.

To further elucidate the influence of chronotype on school performance, we next review the relationship between chronotype and individual/personality factors relevant for school performance.

Chronotype and individual/personality factors relevant for school performance

In addition to chronotype, there are several factors that influence school performance, and some of these factors have also been found to be associated with chronotype. For instance, conscientiousness (being organized, self-disciplined, and goal-oriented) is a personality trait that is positively related to both morningness and high school achievements (Bratko et al., 2006; Randler, 2008; Tonetti, Fabbri, & Natale, 2009). Two recent studies, that investigated both chronotype and conscientiousness in the context of school performance, found that the chronotype-effect on school grades was significant only when conscientiousness was analyzed as mediating factor, suggesting that chronotype influences conscientiousness, which in turn impacts grades (Arbabi et al., 2014; Rahafar et al., 2016).

Roeser et al. (2013) explored the relationship between chronotype, achievement/learning motivation, sleepiness, and school performance. Similarly, they did not find a direct influence of chronotype on school performance, and they concluded that chronotype was likely to influence school performance by increasing sleepiness and decreasing achievement/learning motivation in evening types. In line with this study, Escribano and Díaz-Morales (2016) also found that morning types show higher learning and performance goals, and that these goals are positively associated with school performance. This association was found to be stronger in evening types, suggesting again that the chronotype-effect on grades could be mediated by other factors such as achievement motivation.

Mood, daytime functioning, and alertness are also important for obtaining good grades and are influenced by chronotype (Short et al., 2013; Vollmer et al., 2013; Warner et al., 2008). For instance, Short et al. (2013) showed that evening types have lower sleep quality, increased depressed mood, and decreased alertness. In the same study, students with an evening chronotype and lower sleep quality reported lower grades, and this relationship was mediated by depressed mood.

Finally, intelligence is one of the strongest predictors for school performance (Arbabi et al., 2014; Deary et al., 2007). Despite this, only a few studies have investigated a possible relationship between chronotype and intelligence in adolescents with contradicting results. Arbabi et al. (2014) found that morningness was positively related to intelligence in a sample of 10-year-old children. In older students already attending university, this relationship was reversed with evening types scoring higher on a standardized test for admission to university that correlates with a measure of general intelligence (Piffer, Ponzi, Sapienza, Zingales, &

Maestripieri, 2014). In another study, verbal IQ scores obtained during adolescence were shown to be a predictor of circadian preference, with more intelligent students preferring eveningness as adults (Kanazawa & Perina, 2009). Although not in the context of school performance, the study of Goldstein, Hahn, Hasher, Wiprzycka, and Zelazo (2007) specifically investigated the relationship between chronotype and intelligence, taking into account time of day. Subtests of the WISC-III (Weschler, 1991) were administered to assess both fluid (reasoning, logic, abstract thinking) and crystalized (vocabulary, general knowledge) intelligence at two times of day (morning and afternoon). Only for fluid intelligence there was a significant interaction effect between chronotype and time of day, with morning types obtaining higher scores in the morning and evening types obtaining higher scores in the afternoon. The main effect of chronotype on intelligence scores was not significant, suggesting that chronotype is not associated with intelligence per se, but that the performance in intelligence tests depends on the interaction between chronotype and time of day of testing.

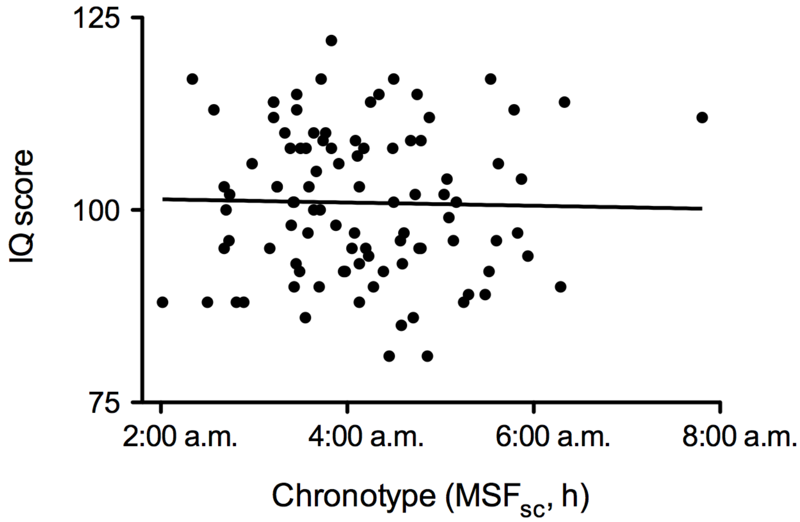

Figure 1. Relationship between chronotype (assessed with the MCTQ) and IQ score (assessed with the NIO) in 97 high school students (54 females, mean age 12.78 ± 1.03 SD). The correlation between chronotype and IQ score was tested with a linear regression model with age and sex as covariates. Chronotype and IQ score were not significantly correlated ($\beta = 0.8901$, $t(93) = 0.844$, $p = 0.4006$). MSF_{sc} = midpoint of sleep on school-free days.

In line with these results, we also found no correlation between chronotype and IQ scores (assessed with the Nederlandse Intelligentietest voor Onderwijsniveau (NIO), a Dutch intelligence test used in schools) in 97 high school students (54 females, mean age 12.78 ± 1.03 SD) when controlling for age and sex (Fig. 1), and when not controlling for time of day (test times were unknown; the Medical Ethical Committee of the University Medical Centre of Groningen (NL), and written informed consent for data utilization was obtained from the head of the school).

In conclusion, there is evidence both for a direct and for an indirect effect of chronotype on grades mediated by other relevant factors for school performance, such as conscientiousness, achieving and learning motivation, mood, and alertness. Figure 2 summarizes the complexity of the influence of chronotype on grades.

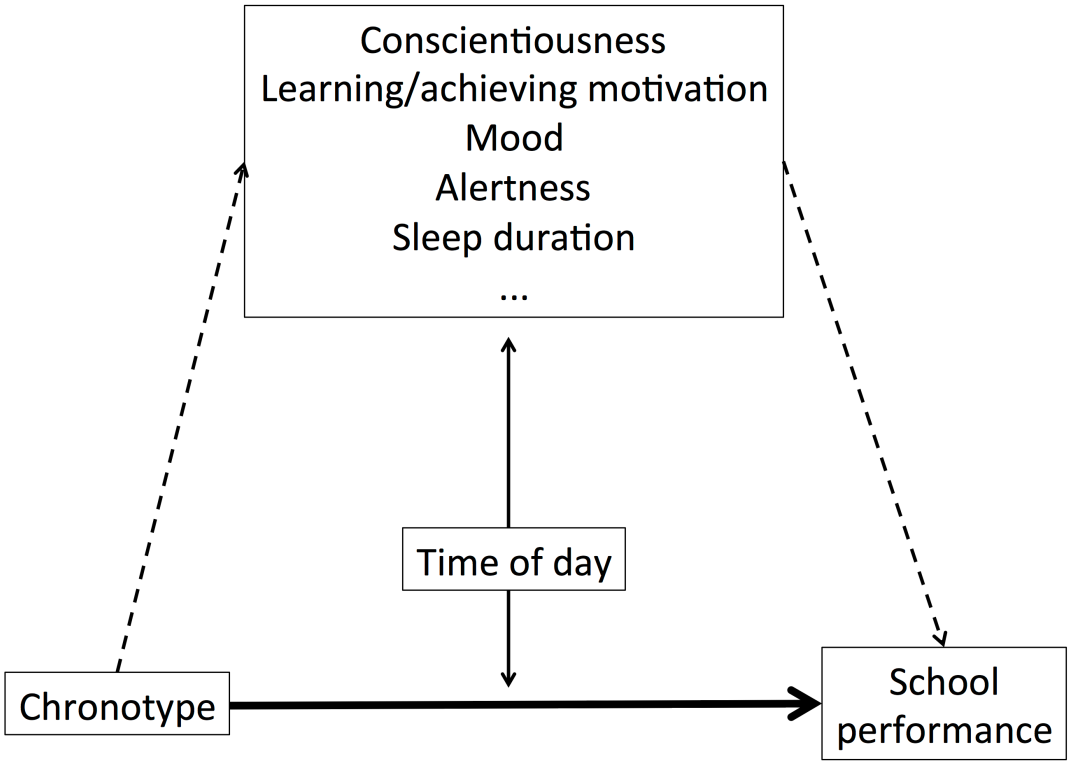

Figure 2. Complexity of the influence of chronotype on school performance.
Chronotype can have both a direct effect (bold solid line) and an indirect effect (dashed lines) on school performance. The direct effect depends on time of day, with time of day acting as moderator variable of the relationship between chronotype and school performance. The indirect effect of chronotype on school performance is mediated by other factors, such as conscientiousness, learning/achieving motivation, mood, alertness, and sleep duration. Chronotype influences these factors, which, in turn, impact school performance. For instance, late chronotypes have lower levels of conscientiousness, and lower levels of conscientiousness are associated with lower school performance. Finally, time of day also influences some of these other factors, such as mood and alertness.

Chronotype, time of day, and school performance

In addition to the factors previously described, time of day should be also taken into account when looking at the effect of chronotype on grades. It is well known that performance at numerous levels shows changes with time of day and is clock regulated. Thus, it is reasonable to hypothesize that the cognitive abilities of different chronotypes vary accordingly with time of day. Several studies have used educational research to investigate the idea.

The first observations that school times might not be optimal for some students were reported in the beginning of the 80's. Both test results and truancy were found to improve when school times were matched to the students' circadian preference (Lynch, 1981; Virostko, 1983). In addition, students who reported feeling more alert and performing better at school in the morning obtained a higher grade point average (Biggers, 1980). In 1999, Callan found that students with a preference for learning in the morning scored better in an algebra test compared to students with a preference for learning in the afternoon and in the evening.

Only recently, a few studies have linked chronotype, time of day, and school performance in adolescents. We showed that the chronotype-effect on grades depends on time of day, with late chronotype underperforming early chronotypes in the morning (8:15h-12:15h), but not in the early afternoon (12:45h-15:00h) (van der Vinne et al., 2015). Similarly, Itzek-Greulich, Randler, and Vollmer (2016) found a chronotype-effect on a chemistry test for students who had attended the course in the morning, with early chronotypes performing better compared with late chronotypes. The association between chronotype and test results was not significant for students who had attended the course in the afternoon. If we assume that early and late chronotypes only differ in their phase of entrainment, these results could be explained with late chronotypes being tested when their peak capacity in cognitive performance has not yet been reached (Fig. 3).
Based on both these findings and also on other results about time-of-day fluctuations in cognitive abilities depending on chronotype, more studies about the effect of chronotype and time of day on school performance are warranted (Escribano & Díaz-Morales, 2014; Goldstein et al., 2007; Hahn et al., 2012; van der Heijden, de Sonneville, & Althaus, 2010).

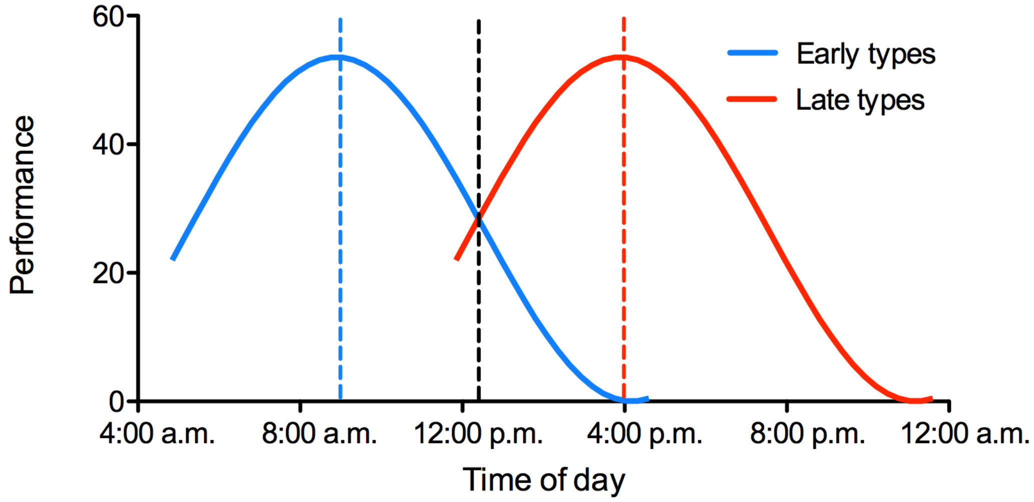

Figure 3. Interaction effect between chronotype and time of day on school grades based on the daily variation in (cognitive) performance in early (blue curve) and late (red curve) chronotypes. Here we assume that the variation in performance has the same shape for early and late chronotypes, but the peak in performance is shifted to a later time point in the day for late chronotypes. The vertical dashed lines indicate when early (blue) and late (red) chronotypes would perform best during an examination. In this particular example early chronotypes would perform best around 9:00 h, late chronotype would perform best around 16:00 h, and no difference between chronotypes (black dashed line) would be observed around 12:30 h.

How to remove the chronotype 'handicap'

During the past decades, several studies have demonstrated that the current school starting times challenge the sleep and performance of high-school students, especially students with late chronotypes. The studies reviewed here show that the influence of chronotype on grades is complex. In general, late chronotypes obtain lower grades, and this can negatively influence their future academic careers (e.g. access to university). There is evidence for a direct effect of chronotype on grades, but also for an indirect effect mediated by other factors relevant for school performance such as conscientiousness, learning/achieving motivation, mood, and alertness. In addition, time of day of testing seems to play an important role acting as a moderator variable of the relationship between chronotype and school grades. Since the poor school performance of late chronotypes arises from a mismatch between the circadian and the social clocks, the efficacy of interventions aiming to reduce this mismatch should be tested in experimental studies (Fig. 4).

Advancing sleep timing in adolescents (especially late chronotypes)

Interventions to advance sleep timing in adolescents, resulting in longer and improved sleep, could be implemented at school. Several sleep hygiene programs have been developed and their efficacy has been tested in randomized-controlled studies. Depending on the specific program, different positive outcomes have been achieved, such as longer sleep duration, earlier bed times, reduced discrepancy between sleep timing on weekdays and on weekends (Kira, Maddison, Hull, Blunden, & Olds, 2014; Moseley & Gradisar, 2009; Wolfson, Harkins, Johnson, & Marco, 2015). However, the observed changes did not always concern all students, and often disappeared at the last follow-up assessments.

The modulation of light exposure at specific times of day could help advancing the late circadian clock of adolescents. As previously described, increased morning light and decreased evening light are two potentially effective interventions. Although the response of the circadian system to light exposure at different times of day is quite well described, only a few studies have tested the effectiveness of light interventions to advance phase of entrainment in adolescents. Concerning morning light, two weeks of white light exposure in classroom (between 8:10 h to 9:43 h) on school days were not enough to shift sleep timing in high-school students (Hansen, Janssen, Schiff, Zee, & Dubocovich, 2005). Similarly, 20-minutes exposure to a dawn simulator before awakening, did not affect sleep timing in adolescents (Tonetti, Fabbri, et al., 2015). One hour of short-wavelength light exposure on weekend days upon awakening was also not effective in counteracting the delay in dim-light melatonin onset over the weekend (compared to weekdays) in adolescents (Crowley & Carskadon, 2010). Although these results suggest that morning light might not be effective in advancing sleep of adolescents, it is possible that timing and intensity of light exposure were not optimal. It is also possible that adolescents are less sensitive to light as a zeitgeber (Roenneberg et al., 2015).

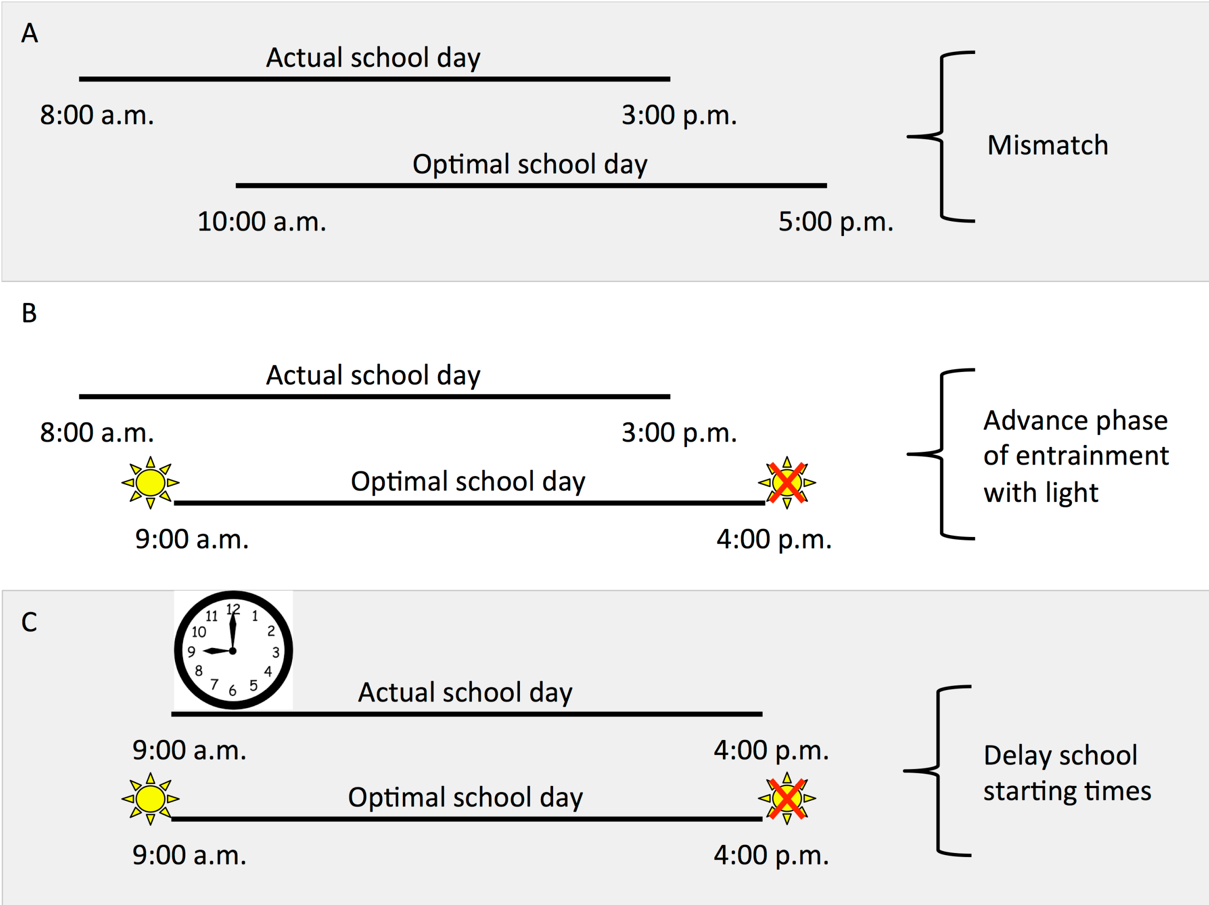

Figure 4. Strategies to optimize the school day for adolescents.
(A) The early school starting times and the late circadian clock of adolescents give rise to a mismatch that has consequences for sleep, and performance of the students. (B) One way to decrease this mismatch is to apply interventions to advance sleep timing in adolescents as, for example, increasing light exposure in the morning and/or decreasing light exposure in the evening. (C) In addition to advancing the phase/chronotype of students using light (as in panel B), school starting times could be delayed, thus decreasing the mismatch using social and biological considerations.

The use of technology in the evening (computers, phones, video games) with, as a consequence, an increased exposure to evening (blue) light was listed as a risk factor for adolescent sleep in a recent meta-analysis (Bartel, Gradisar, & Williamson, 2015). A simple intervention, such as wearing blue-light-blocking glasses in the evening, was shown to reduce the suppression of melatonin associated with exposure to light, and to decrease alertness before bedtime (van der Lely et al., 2015). Similar results in terms of decreased alertness were found when comparing the use of a bright screen with a dim screen 1 hour before bedtime (Heath et al., 2014). In both studies, no significant changes in sleep variables (e.g. sleep onset latency) were found, although several studies have shown an association between the use of media in the evening and sleep disturbances (Cain & Gradisar, 2010; Munezawa et al., 2011; van den Bulck, 2004). More research is needed to establish guidelines about the evening use of electronic devices in adolescents in relation to their sleep.

Other solutions to improve sleep in adolescents could involve physical activity. A recent study showed that running for 30 minutes in the morning on a school day (for 3 weeks) shortened sleep latency, improved sleep quality, and increased time spent in deep sleep (Kalak et al., 2012). In this study morning light exposure was controlled for, suggesting that the positive effect on sleep was a result of morning physical activity.

Taken together, although there is evidence for a delayed, short, and disturbed sleep in adolescents, there are too few studies testing interventions to advance sleep, and to increase sleep duration and quality in adolescents. The light interventions previously described are supposed to influence the phase of entrainment of the circadian clock. The homeostatic process, also involved in the regulation of sleep, has received less attention, although one of the hypotheses for a delay in sleep timing in adolescents is a decreased susceptibility to the build-up of sleep pressure during the day (Hagenauer et al., 2009; Jenni et al., 2005). Future research should extensively test different light interventions to advance sleep timing in adolescents, trying to elucidate which intensities and which type of light (e.g. color) to apply/avoid at particular times of day. This should be specifically tested in adolescents since their sensitivity to light might be different from adults (Roenneberg et al., 2015). In addition, interventions that could affect sleep pressure should be also developed and tested.

Delaying school starting times

In addition to strategies to advance sleep timing, another solution to improve school performance would be to delay school starting times. Not only late chronotypes, but also the majority of high-school students, would benefit from a delay in school starting times. In fact, several studies have reported worrying statistics about sleep duration (on a typical school night) in adolescents, which is shorter than the recommended 9 hours of sleep for about 80-90 % of the students (C. E. Basch, Basch, Ruggles, & Rajan, 2014; Gibson et al., 2006; R. E. Roberts, Roberts, & Duong, 2009). Interestingly, already in 1913, Terman and Hocking hypothesized that the longer sleep duration observed in American compared to German adolescents was in part due to a later school starting time in the US (US at 9:00 h vs. Germany at 8:00 h). Recently, Wolfson and Carskadon (2005) published a survey about the factors influencing high-school starting times. Urban schools and schools with larger enrollments tended to start earlier. The socio-economic status of the parents was also found to be associated with school starting times, with higher status related to earlier starting times. Finally, schools starting times correlated with (trivial) factors such as how bus schedules were organized.

In the past decades, several schools have delayed their school starting times. There is increasing evidence of positive outcomes in terms of mood, sleep, daytime sleepiness, school attendance, and school performance after a delay in school starting times was introduced (Boergers, Gable, & Owens, 2014; Carrell, Maghakian, & West, 2011; Owens, Belon, & Moss, 2010). For a complete overview of schools that have successfully implemented later school schedules we suggest to read the work of Owens, Drobnich, Baylor, and Lewin (2014) and of Wahlstrom et al. (2014). Still, more longitudinal studies monitoring the effects of delayed school starting times are needed. For instance, a recent study showed that a 45-minutes delay in school starting times was associated with a 20-minutes gain in sleep duration (within the first 6 months after the change) that was lost after 1 year (Thacher & Onyper, 2016).

Two other studies have assessed differences in sleep between high-school students attending morning or afternoon lectures, taking chronotype into account (Koscec, Radosevic-Vidacek, & Bakotic, 2013; Martin, Gaudreault, Perron, & Laberge, 2016). In general, sleep duration was significantly longer for students attending the afternoon shifts. This was observed even in morning types who also reported higher sleepiness during the morning shift (Martin et al., 2016). Although in a small sample of students (N=57), the authors found no significant difference in grades between students attending the morning and the afternoon shift, and overall evening types did not obtain lower grades compared to morning types. These studies suggest that early school starting times (7:40 h and 8:00h) might have detrimental effects even for students classified as 'morning types' on the MEQ scale.

Some schools, for instance in The Netherlands, have also experimented with flexible starting times: core subjects are taught in the middle of the day, while other school activities are offered earlier in the morning or later in the afternoon, and the students can choose their preferred time for attending these activities (http://www.deschool.nl).
More studies are needed to assess whether students' sleep and performance could benefit from such a flexible school system. In a recent study, the widely described chronotype-effect on grades was not found among online-learning students, suggesting again that self-selected sleep and learning schedules could improve students' performance (Horzum, Önder, & Beşoluk, 2014).

Changing school schedules

If a delay in school starting times is not feasible, another solution could involve a rearrangement of school schedules. For instance, the finding that the chronotype-effect on grades depends on time of day of testing advocates that tests should be scheduled later in the day (Itzek-Greulich, Randler, & Vollmer, 2016; van der Vinne et al., 2015). Already in 1995, Callan suggested that an important test for access to college such as the SAT (Scholastic Aptitude Test) should be offered at different times of day (and not only at 8:30 h) in order to not discriminate students according to their time-of-day preferences in learning. Considering time of day is also essential for scheduling the different school subjects within the school day. As reviewed by Wile and Shouppe (2011), there is a time-of-day dependency in specific cognitive abilities necessary for different school subjects, and this is likely to vary between chronotypes. Although more research is needed to establish the optimal time of day for teaching and testing, many schools have already begun changing their schedules.

Conclusion

To allow late chronotypes to perform at their best, schools could take advantage of the recent findings reviewed here. For instance, late chronotypes seem to particularly suffer in specific areas related to learning and school performance, such as conscientiousness and motivation

(Arbabi et al., 2014; Rahafar et al., 2016; Roeser et al., 2013). With the use of simple questionnaires such as the MCTQ and the MEQ, late chronotypes could be first identified, and then interventions could be developed to increase their levels of conscientiousness and motivation, with a possible positive outcome in terms of school performance.

Many studies are still needed to fully understand the circadian system of adolescents, to unravel the mechanisms behind the effects of chronotype on school performance, and to develop effective strategies to improve school performance in late chronotypes. Nonetheless, the iterative exchange of data between schools and scientific researchers is setting up the basis for a more fair school system for all students, independent of their chronotype.

Chapter 6

Light interventions to decrease social jetlag

Giulia Zerbini, Thomas Kantermann, Till Roenneberg, and Martha Merrow

Abstract

Adequate and efficient sleep is essential for good health and peak performance. The circadian clock is involved in the regulation of sleep timing and efficient sleep is only possible within a certain sleep window. Due to genetic, developmental and environmental differences between individuals, a broad distribution in sleep timing is observed in populations. In contrast to the richness of circadian clock-mediated sleep timing (chronotype), school and working schedules tend to be uniform across social life. Especially late chronotypes - those who sleep late - suffer from a phenomenon called social jetlag (SJL; mismatch between the circadian/biological and social clocks). SJL has been related to several health issues, yet no intervention to decrease it has been tested so far.

If we accept that the major determinants of chronotype are genetics, age and light environment, it is obvious that active modification of chronotype is only possible by using light. Indeed, light is the most important zeitgeber for human behavioral rhythms, keeping the sleep/wake cycle synchronized (entrained) to the external light-dark cycle. Exposure to light (especially blue light) in the early biological night (evening) delays the clock, while exposure to light in the late biological night (morning) advances it. We developed two *in situ* protocols to advance sleep timing and phase of entrainment. In Study 1, evening light exposure was decreased by wearing blue-light-blocking glasses in the evening. In Study 2, morning light exposure was increased by sleeping with open curtains. Our measures were sleep timing (via sleep diaries), activity timing (via actimetry), and entrained phase (via dim-light melatonin onset; DLMO). We found that a decrease in evening light exposure was associated with an advance in sleep onset and in DLMO on workdays (36 and 32 minutes respectively). The increase in morning light exposure did not yield the same results. However, the participants who experienced a greater increase in bedroom light intensities (by sleeping with open curtains) showed the biggest advances in DLMO. In both studies, there was no significant change in SJL. More studies are warranted to determine whether SJL could be decreased by light and whether this would benefit late chronotypes in terms of health and performance.

Introduction

Sleep is a basic human need, important for health and performance. A two process model of sleep regulation suggests a juxtaposed homeostatic (sleep pressure) and circadian process (Borbély, 1982; Daan, Beersma, & Borbély, 1984). The circadian clock actively synchronizes (entrains) to the external light-dark cycle with a specific phase relationship (Duffy & Wright, 2005; Roenneberg & Merrow, 2007; Wright et al., 2013). The variation in genetic background, sex, and age together with the variation in daily light exposure leads to a distribution of entrained phases relative to the light-dark cycle (Hamet & Tremblay, 2006; Roenneberg et al., 2007a; Roenneberg, Kumar, & Merrow, 2007b; Wright et al., 2013). Chronotype is generally measured with questionnaires (e.g Munich ChronoType Questionnaire (MCTQ), Roenneberg, Wirz-Justice, & Merrow, 2003; Morningness-Eveningness Questionnaire (MEQ), Horne & Ostberg, 1976). With the MCTQ, chronotype is assessed as the midpoint of sleep on work-free days (MSF) with a correction for sleep debt accumulated on workdays (MSF_{sc}). We use the MCTQ because we can additionally obtain detailed information about sleep timing (separately for workdays and work-free days).

The substantial individual differences in sleep timing are often neglected in modern society when, for example, standardized social programs are imposed broadly, such as school opening and work times. This leads to a phenomenon named social jetlag (SJL; Wittmann, Dinich, Merrow, & Roenneberg, 2006). SJL represents the mismatch between the circadian and social clocks and is assessed as the absolute difference between the midpoint of sleep on workdays (MSW) and on work-free days (MSF). SJL is usually greater in late chronotypes, with late chronotypes typically being sleep deprived during the school/working week (waking up earlier than the clock would specify and requiring the use of an alarm clock) and sleeping longer and later on work-free days. In most cases of SJL, MSF is later than MSW.

In previous studies, SJL has been significantly associated with several health issues, such as increased nicotine addiction/smoking, alcohol consumption and obesity (Roenneberg, Allebrandt, Merrow, & Vetter, 2012; Wittmann et al., 2006). Here, we aimed to reduce SJL by using light interventions, given that light is the strongest zeitgeber for human behavioral entrainment. Our goal was to advance the sleep timing of late chronotypes and better match it to the demands of early social schedules.

Light intensity, spectral quality and the timing of exposure are utilized by the circadian clock for entrainment (Duffy & Wright, 2005). For instance, when exposed to light pulses at different times of day, humans can respond with advancing or delaying phase shifts (Khalsa, Jewett, Cajochen, & Czeisler, 2003). In particular, light exposure during the beginning of the biological night induces phase delays and light exposure during the end of the biological night induces phase advances (Khalsa et al., 2003). Concerning spectral quality, the circadian response to light is most sensitive to blue light (Brainard et al., 2001). Changing the light intensities in a home setting was shown to influence phase of entrainment (estimated via dim-light melatonin onset; DLMO), with later DLMOs associated with higher evening ambient light intensities (Burgess & Molina, 2014). Another study demonstrated that controlling morning and evening light exposure was more important than changing sleep timing in influencing DLMO (Appleman, Figueiro, & Rea, 2013). Based on these studies, we developed two *in situ* protocols to advance phase of entrainment in late chronotypes with a relatively high SJL. In Study 1, evening blue light exposure was decreased using orange (blue light blocking) glasses. In Study 2, morning light was increased simply by keeping windows unobstructed. We hypothesized that both protocols (less evening light and more morning light) would advance sleep timing and phase of entrainment, leading to a longer sleep duration on workdays, a consequent reduction of oversleep on work-free days, and a decrease in SJL (Fig. 1). In both studies, we aimed to test the effectiveness of practical interventions in shifting sleep timing and phase of entrainment with the aim of a direct applicability of our findings in real life conditions.

We found that wearing blue-light-blocking glasses in the evening was effective in advancing sleep timing (on workdays) and phase of entrainment (DLMO), while sleeping with open curtains did not yield similar results. However, the strength of the intervention (amount of increase in morning bedroom light intensities) was correlated with the degree of shift in DLMO in the expected direction (more light was associated with a greater advance).

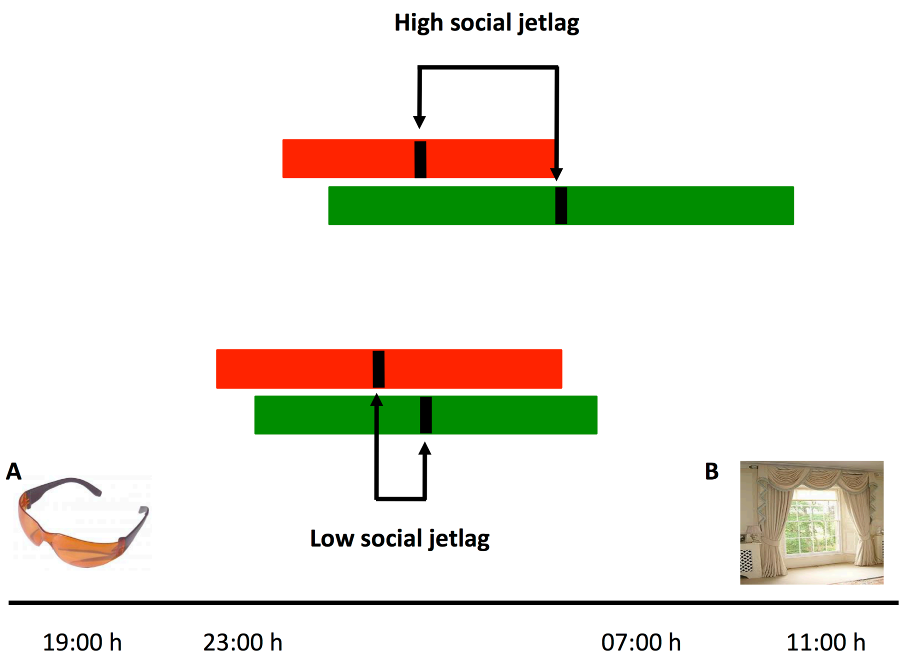

Figure 1. Decreasing social jetlag (SJL) with light.
The bars represent sleep (red bars on workdays and green bars on work-free days). The vertical black lines represent the midpoint of sleep on workdays (MSW) and on work-free days (MSF). SJL is calculated as the absolute difference between MSW and MSF. The two light interventions to decrease SJL involve wearing blue-light-blocking glasses in the evening (A) and sleeping with open curtains (B). Both interventions are expected to advance sleep timing and phase of entrainment, leading to a reduction of sleep debt accumulated on workdays and, as a consequence, also a reduction of oversleep on work-free days. This should result in a decrease of SJL via a better alignment of MSW and MSF.

Methods

Study 1 – evening (blue) light

Participants

The study was run in February 2015 in Groningen (53°13′ N / 6°33′ E), The Netherlands. Participants were recruited via flyer and online advertisement. A total of 40 participants (24 females) were selected for the study, and 38 (23 females) between the ages of 19 and 47 (mean age 23.7 ± SD 5.5) completed it. Participants were generally healthy, had no sleep complaints, and they did not make use of any medication. Participants had a regular working schedule (at least 4 working days per week), had not performed shift work during the past 5 years, and had not travelled across more than 2 time zones during the month prior to the study. Females were selected only if they made use of hormonal contraceptives (to avoid possible fluctuations in melatonin levels depending on the phase of the menstrual cycle; Lee Barron, 2007). Participants had at least 1.5 hours of SJL assessed via the Munich Chronotype Questionnaire (MCTQ; Roenneberg et al., 2003) as the absolute difference between the midpoint of sleep on work-free days (MSF) and the midpoint of sleep on workdays (MSW) (Wittmann et al., 2006).

Protocol

The study lasted 4 weeks (from 02.02.2015 to 01.03.2015). The participants were randomly assigned to one of two groups: the control group and the intervention group. The groups were matched for age, sex, chronotype (the sleep corrected midpoint of sleep on work-free days; MSF_{sc}) and SJL.

After two weeks of baseline, participants wore a special pair of glasses every evening for the remaining two weeks of the study. The control group wore glasses with clear lenses (no filter of blue light between 400-500 nm, general decrease of light intensity: 8%; for more details see Fig. S1 in the Supplementary Information). The intervention group wore glasses with blue-light-blocking lenses (89-99,9% filter of blue light between 400-500 nm, general decrease of light intensity: 50%; for more details see Fig. S2 in the Supplemental Information). The participants started wearing the glasses 9 hours before their chronotype (MSF_{sc}) until they turned the lights off to sleep. In this way, we aimed to apply the intervention at the same internal time (internal phase) for all participants.

Study 2 - morning (natural) light

Participants

The study was conducted in March 2016 in Groningen (53°13′ N / 6°33′ E), The Netherlands. Participants were recruited via flyers, online advertisements, and internal posting at the University of Groningen. The same inclusion and exclusion criteria (general health, regular working schedule, no shiftwork or travelling across time zones) were applied to select the participants as for Study 1. In addition, only people who habitually slept with dark closed curtains could sign up for the study. The latest 40 chronotypes of the eligible applicants (22 females, mean age 22.6 ± SD 3.1, range 18-35) were selected for the study. 38 participants (20 females, mean age 22.8 ± SD 3.1, range 19-35) completed the study.

Protocol

The study lasted 30 days (from 26.02.16 to 26.03.16). Participants were randomly assigned to the control or the intervention group. The two groups were matched for sex, age, chronotype, and SJL. The control group slept with curtains closed throughout the protocol. The intervention group slept with curtains closed for 10 days (baseline), then for 14 days with curtains open (intervention weeks), and again with curtains closed for 7 days (wash-out week). After baseline and after the intervention weeks the participants filled in the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) to assess whether sleeping with open curtains influenced subjective sleep quality. The two protocols are shown in Figure 2.

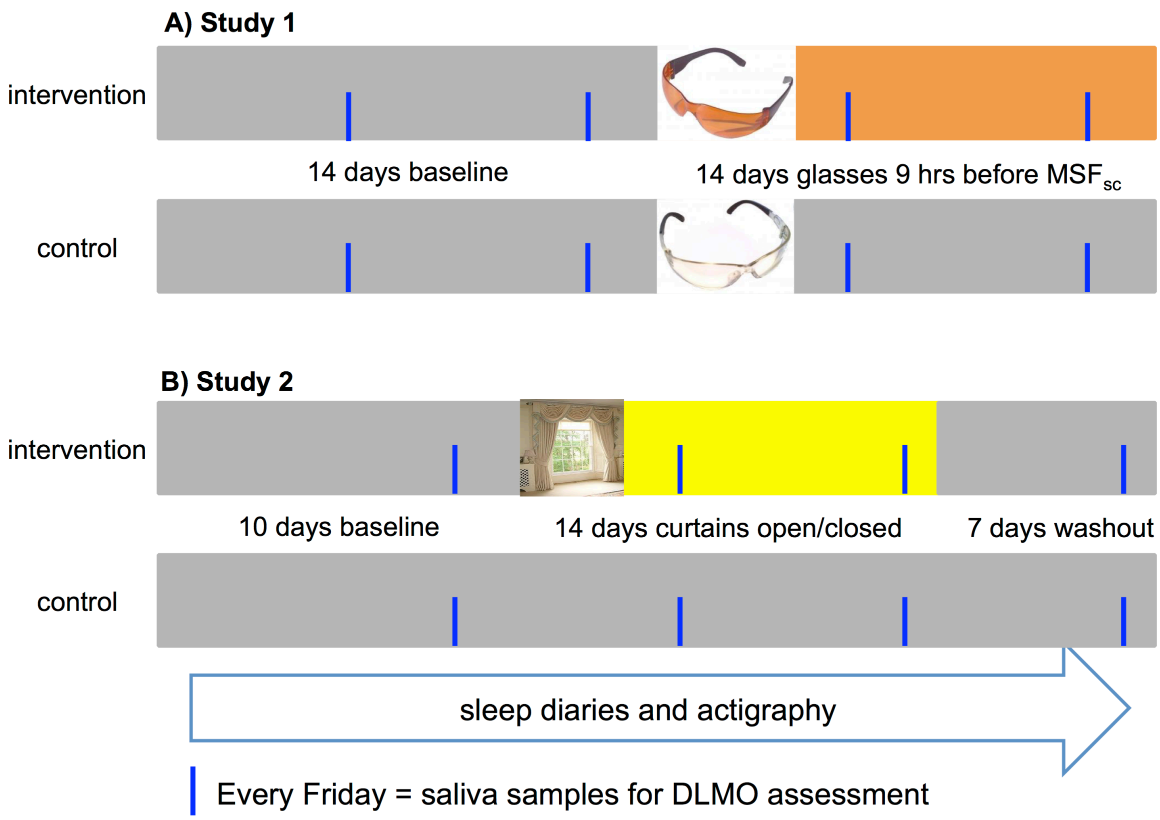

Figure 2. Experimental design.
A) Protocol used in Study 1. After 2 weeks of baseline (grey blocks), the intervention group started wearing the orange (blue-light-blocking) glasses and the control group the glasses with clear lenses for 2 weeks. B) Protocol used in Study 2. After 10 days of baseline (grey blocks), the intervention group slept with bedroom curtains open for 2 weeks and after that with bedroom curtains closed for 1 week. The control group slept with bedroom curtains closed throughout the study.
In both studies, participants collected saliva samples every Friday of the protocol (4 times), filled in a sleep diary, and wore continuously an actiwatch (for light and activity measurements). The evenings of saliva sample collection are indicated with vertical blue lines.

Study 1 and 2

Sleep, activity, and light assessment

During both protocols, the participants filled in a daily sleep diary and continuously wore an actiwatch (Study 1: Daqtometer Version 2.3, Daxtix GbR, Suettorf, DE; Study 2: MotionWatch 8, CamNtech, Cambridge, UK). The actiwatches recorded both activity and light intensity levels. Actigraphy data were analyzed with ChronoSapiens (version 9). In Study 2, participants also used a light sensor (HOBO pendant temperature/light 64K data logger, Onset, Bourne, MA, US) in the bedroom to assess light intensities throughout the study.

Circadian phase assessment (DLMO)

Circadian phase was estimated by assessing dim-light melatonin onset (DLMO) from saliva samples. The participants collected 7-hourly saliva samples every Friday evening (if Friday was not possible, either Thursday or Saturday were allowed for the saliva sample collection). The saliva sample collection was individually timed, 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). The saliva sample collection took place at the participants' home. Participants were requested to dim their home lighting as much as possible, and to start wearing a pair of blue-light blocking glasses half an hour before the collection of the first sample until the collection of the last sample. During each evening of saliva samples collection, the use of toothpaste and the ingestion of coffee, tea, alcohol, chocolate, banana, and food with artificial additives were not allowed. The saliva samples were collected using Salivettes (Sarstedt, Nümbrecht, DE). The samples were kept in the fridge and sent per mail to the lab within 3 days. Upon arrival, the samples were frozen at –80 °C and subsequently analyzed using direct saliva melatonin radioimmunoassay (RIA) test kits (Bühlmann, Schönenbuch, CH). 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. In study 1, the intra-assay variability was 19.81% (low melatonin) and 22.13% (high melatonin), while the inter-assay variability was 14.67% (low melatonin) and 16.54% (high melatonin). In study 2, the intra-assay variability was 12.60% (low melatonin) and 16.18% (high melatonin), while the inter-assay variability was 14.72% (low melatonin) and 13.25% (high melatonin).

Statistical analysis

Statistical analyses were done using R software (R version 3.3.0; The R Core team, 2013). Data about sleep timing, activity and DLMO were analyzed with a mixed within-between model with simple planned comparisons (to baseline). A 2 (group: control vs intervention) x 3 (time: baseline vs first intervention week vs second intervention week) design was used for the analyses in Study 1. A 2 (group: control vs intervention) x 4 (time: baseline vs first intervention week vs second intervention week vs wash-out week) design was used for the analyses in Study 2. In Study 1, morning light (from 6:00 h till 12:00h) was analyzed as a covariate to control for the advancing effects that also morning light potentially has on sleep and phase of entrainment. In Study 2, evening light (from 18:00 till 0:00) was analyzed as a covariate to control for the delaying effects that evening light potentially has on sleep and phase of entrainment.

If the interaction effect between group and time was significant, the change in the variables of interest during the first intervention week and the second intervention week (and the wash-out week in Study 2) relative to baseline was analyzed comparing the two groups (control vs intervention) with one-tailed independent t tests (Bonferroni correction was applied for multiple comparisons). In addition, in Study 2 the changes in bedroom light intensities and in sleep quality (PSQI) between baseline and during/after the intervention were compared between the two groups with a Mann-Whitney-Wilcoxon test.

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

Results

Study 1 – evening (blue) light

The demographics of the participants are reported in Table 1. The two groups had the same ratio of female and male participants. Independent t tests were run to confirm that control and intervention groups did not differ at baseline in terms of age, chronotype, and SJL (age: $t(36) = -0.116$, $p > .05$; chronotype: $t(36) = -1.312$, $p > .05$; SJL: $t(36) = -0.334$, $p > .05$).

In Figure 3 the light profiles for the two groups during baseline, and during first and second intervention weeks are shown. Overall, participants were exposed to comparable light levels, and therefore any change observed in the variables of interest is likely to be related to wearing the blue-light-blocking glasses and not to differences in light exposure between groups and across the protocol.

The analysis of the variables assessed with the daily sleep diaries revealed significant changes in sleep on workdays but not on work-free days. In all analyses morning light (between 6:00 h and 12:00 h) was analyzed as a covariate. In particular, we found a significant interaction effect between group and time on sleep onset on workdays ($F_{2,39.14} = 3.653$, $p = 0.0351$), indicating differences between the control and the intervention groups across the protocol. To explore in which weeks of the protocol the two groups differed, we compared the change in sleep onset on workdays during the first and the second intervention week (relative to baseline) between the groups (Fig. 4). The intervention group showed an advance in the sleep onset on workdays (on average 36 minutes) during the first intervention week relative to baseline. This advance was significant compared to the control group ($t(36) = -2.606$, $p = 0.0133$). The effect size was large (eta squared = 0.16) according to Cohen's guidelines (0.01

= small effect; 0.06 = medium effect; 0.14 = large effect). Although the intervention group showed an earlier sleep onset also during the second intervention week relative to baseline (on average 18 minutes), the effect was not significant anymore when compared to the control group ($t(35) = -1.136$, $p > .05$).

Table 1. Demographics of 38 (23 females) participants (Study 1) randomly assigned to the control and the intervention group (data from the Munich ChronoType Questionnaire).

	Control group		Intervention group	
Parameter	Average (SD)	Range	Average (SD)	Range
Age	23.63 (7.00)	19 - 47	23.84 (3.72)	19 - 33
Chronotype (MSF_{sc}, h)	5.02 (1.04)	3.17 - 7.20	5.49 (1.15)	3.51 - 8.40
Social Jetlag (h)	2.07 (0.51)	1.46 - 3.46	2.12 (0.47)	1.58 - 3.46
Sleep onset on workdays (h)	-0.29 (0.84)	-1.58 - 1.25	-0.13 (1.14)	-1.83 - 2.67
Sleep end on workdays (h)	7.19 (0.80)	5.75 - 8.50	7.62 (1.21)	5.17 - 9.42
Sleep duration on workdays (h)	7.48 (0.87)	5.17 - 9.42	7.75 (0.88)	5.25 - 9.33
Sleep onset on work-free days (h)	1.05 (1.09)	-1.08 - 3.25	1.45 (1.25)	-0.33 - 4.17
Sleep end on work-free days (h)	9.97 (0.92)	8.00 - 12.00	10.28 (1.28)	8.25 - 13.00
Sleep duration on work-free days (h)	8.92 (0.67)	7.42 - 10.08	8.82 (0.90)	7.17 - 10.5

The two groups did not significantly differ in relation to their demographic and sleep characteristics at baseline. 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).

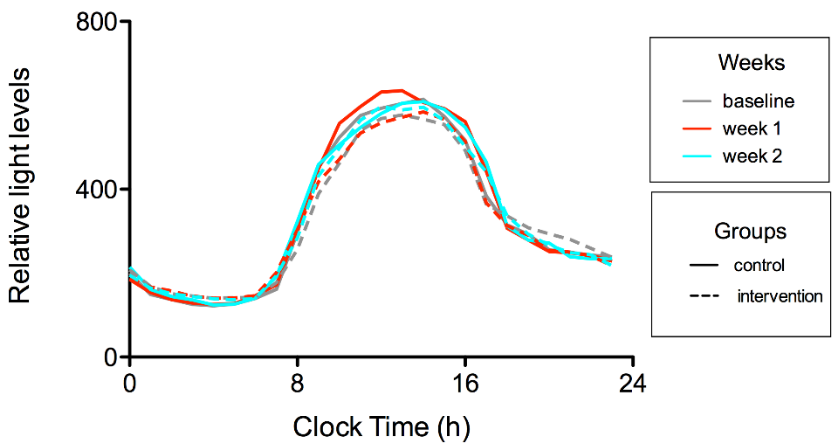

Figure 3. Light profiles of the control and intervention groups across the weeks of the protocol.
The average light intensities (relative light levels) in bins of 1 hour were calculated during the two weeks of baseline (grey), the first (red) and second (light blue) intervention week, separately for the control (solid line) and intervention (dashed line) groups. The data do not show evident differences in light exposure between the groups across the weeks of the protocol.

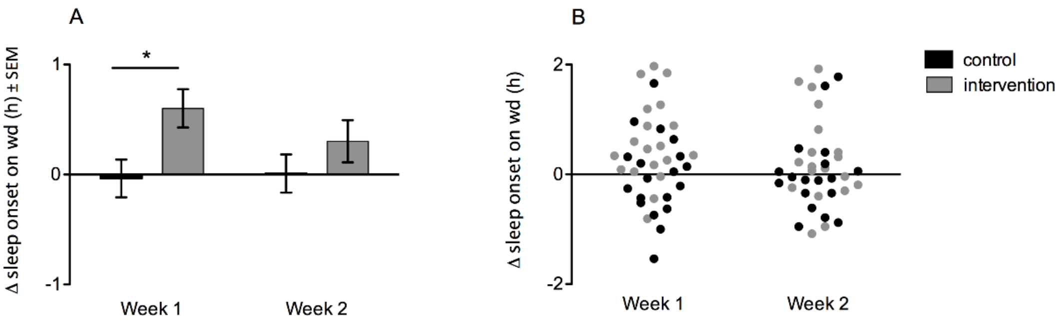

Figure 4. Shift in sleep onset on workdays relative to baseline.
Both group averages with standard error of the mean (A) and individual data points (B) are plotted separately for the control (black) and the intervention (grey) groups. The changes in sleep onset during the first and second intervention weeks are plotted relative to baseline. Positive values represent phase advances and negative values phase delays. During the first intervention week (relative to baseline), the intervention group significantly advanced the sleep onset on workdays (on average 36 minutes) compared to the control group (*p < .05 with Bonferroni correction).

There was a trend for an interaction effect between group and time on DLMO ($F_{2,25.447}$ = 3.001, p = 0.0676). As for sleep onset on workdays, we compared the shift in DLMO during first and second intervention week (relative to baseline) between the two groups (Fig. 5). Compared to the control group, the intervention group showed a significant advance in DLMO (on average 32 minutes) during the first intervention week (t (24) = -2.402, p = 0.0244), but not during the second intervention week (t (22) = -0.388, p > .05). As for sleep onset, the effect size during the first intervention week was large (eta squared = 0.22).

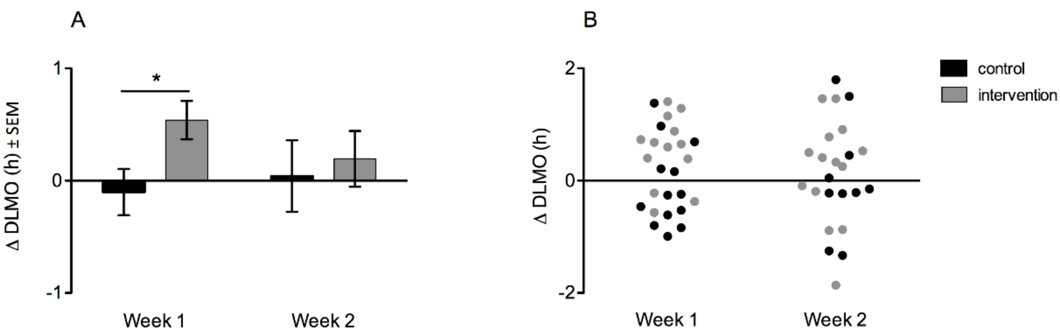

Figure 5. Shift in dim-light melatonin onset (DLMO) relative to baseline.
Both group averages with standard error of the mean (A) and individual data points (B) are plotted separately for the control (black) and the intervention (grey) groups. The changes in DLMO during the first and second intervention weeks are plotted relative to baseline. Positive values represent phase advances and negative values phase delays. During the first intervention week (relative to baseline), the intervention group significantly advanced DLMO (on average 32 minutes) compared to the control group (*p < .05 with Bonferroni correction).

We did not find any significant interaction effect for sleep end and sleep duration on workdays (sleep end: $F_{2,42.23} = 0.669$, $p > .05$; sleep duration: $F_{2,27.11} = 1.049$, $p > .05$). Still, there was a trend indicating that the intervention group slept longer (on average 17 minutes) compared to the control group during the first intervention week relative to baseline ($t(36) = 1.873$, $p = 0.0692$). SJL did not significantly change between groups and across the protocol ($F_{2,26.79} = 0.689$, p >. 05). Finally, center of gravity (a phase marker that can be derived from actigraphy data) also did not significantly change both on workdays and on work-free days (workdays: $F_{2,32.70} = 0.221$, $p > .05$; work-free days: $F_{2,153.69} = 0.802$, $p > .05$).

Study 2 - morning (natural) light

The demographics of the participants are reported in Table 2. In the control group there were 11 females and 9 males, while in the intervention group there were 9 females and 9 males (2 dropouts in the intervention group). Independent t tests were run to confirm that control and intervention groups did not differ at baseline in terms of age, chronotype, and SJL (age: $t(36) = -0.130$, $p > .05$; chronotype: $t(36) = -1.076$, $p > .05$; SJL: $t(36) = 0.047$, $p > .05$).

Table 2. Demographics of 38 (20 females) participants (Study 2) randomly assigned to the control and the intervention group (data from the Munich ChronoType Questionnaire).

	Control group		Intervention group	
Parameter	Average (SD)	Range	Average (SD)	Range
Age	22.70 (3.45)	19 - 35	22.83 (2.81)	19 – 29
Chronotype (MSF_{sc}, h)	5.37 (0.53)	4.70 – 6.40	5.59 (0.73)	4.71 – 7.80
Social Jetlag (h)	1.67 (0.66)	0.63 – 2.96	1.66 (0.67)	0.71 – 2.92
Sleep onset on workdays (h)	-0.21 (0.62)	-1.42 – 0.71	0.25 (0.68)	-0.92 – 1.75
Sleep end on workdays (h)	8.00 (0.86)	7.00 – 10.00	8.20 (0.55)	7.50 – 9.00
Sleep duration on workdays (h)	8.21 (0.84)	6.29 – 9.75	7.95 (0.71)	7.15 – 9.92
Sleep onset on work-free days (h)	1.22 (0.55)	0.50 – 2.30	1.51 (0.91)	0.08 – 4.00
Sleep end on work-free days (h)	9.91 (0.83)	9.00 – 12.00	10.26 (0.90)	9.00 – 12.50
Sleep duration on work-free days (h)	8.69 (0.83)	7.33 – 10.50	8.76 (0.72)	7.25 – 9.98

The two groups did not significantly differ in relation to their demographic and sleep characteristics at baseline. 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).

We first compared the light intensities in the bedrooms during the intervention weeks (relative to baseline) between the two groups. Sleeping with open curtains significantly increased the morning light levels (first 2 hours after dawn) in the bedrooms of the intervention group compared to the control group (U = 42, z = -3.664, p = 0.0001; Fig. 6). The intervention did not negatively affect subjective sleep quality (U = 106.5, z = 0.420, p > .05).

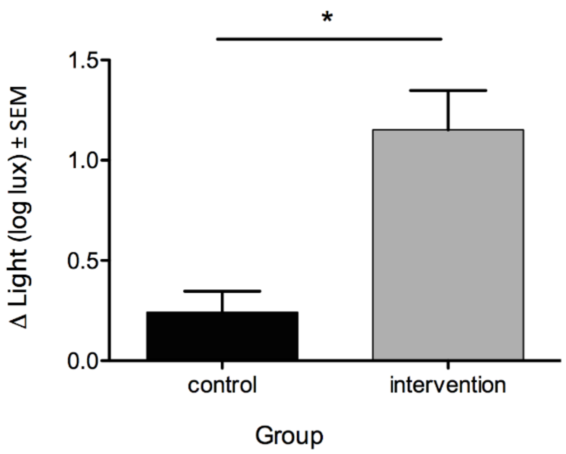

Figure 6. Change in bedroom light intensities relative to baseline.
The bars represent the average light intensities with standard error of the mean (log lux) recorded in the bedrooms during the intervention weeks relative to baseline for the control (black) and the intervention (grey) groups. Light intensities during the 2 hours after dawn are plotted. During the intervention weeks, for the participants who slept with open curtains (intervention group), a significant increase in morning bedroom light intensities was observed as compared to the control group. (* p < .05).

Although the intervention was effective in increasing the morning bedroom light intensity, the two groups did not differ in terms of morning light exposure (between 6:00 h and 12:00 h) across the weeks ($F_{3,34.036} = 1.208$, p > .05; Fig. 7). Similarly, we did not find any significant change between groups in all the variables assessed with the sleep diaries and actigraphy both on workdays and on work-free days. SJL and DLMO also did not significantly change between groups across the study (SJL: $F_{3,59.736} = 1.991$, p > .05; DLMO: $F_{3,53.016} = 0.856$, p > .05). However, the interaction effect between the change in bedroom light intensities and time (week of protocol) on DLMO was significant ($F_{3,37.929} = 3.2410$, p = 0.0326). The shift in DLMO during the first intervention week (relative to baseline) was significantly correlated to the change in bedroom light intensity in the intervention group (b = 0.832, t (13) = 2.711, p = 0.0178, R^2 = 0.36; Fig. 8A). In other words, the strength of the intervention (increase in morning bedroom light intensity) was associated with the change in DLMO, with greater advances in the participants whose bedrooms received more morning light during the intervention. A similar correlation was found during the second intervention week, but this was not significant (b = 0.557, t (13) = 1.635, p = 0.126; Fig. 8B).

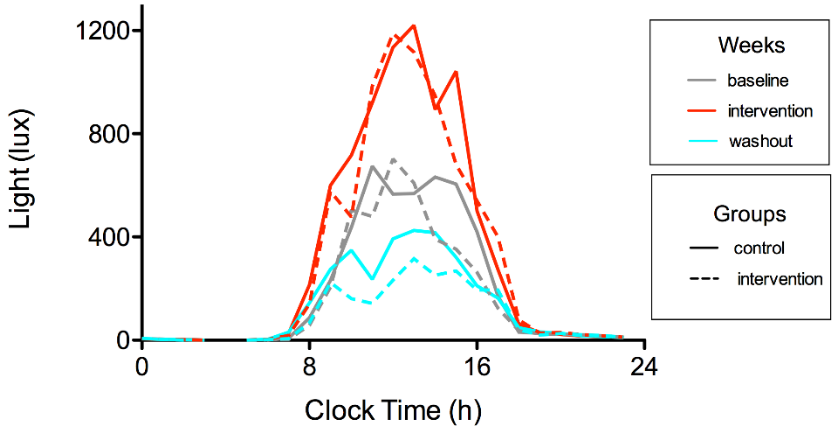

Figure 7. Light profiles of the control and intervention groups across the weeks of the protocol.
The average light intensities (lux) in bins of 1 hour were calculated during the baseline (grey), the two intervention weeks (red), and the washout (light blue), separately for the control (solid line) and intervention (dashed line) groups. The data do not show evident differences in light exposure between the groups. Both groups were exposed to more light throughout the day during the intervention weeks.

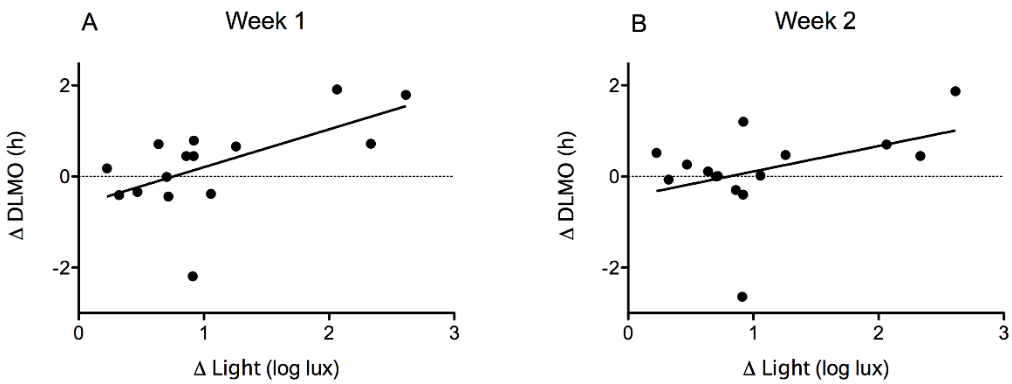

Figure 8. Correlation between change in dim-light melatonin onset (DLMO) and in bedroom light intensities.
The correlation between the shift in DLMO and the change in morning bedroom light intensities during the first (A) and the second (B) intervention weeks relative to baseline is plotted for the intervention group only. There was a positive significant (only during the first intervention week) correlation, indicating that the participants who experienced a greater increase in morning bedroom light intensities showed a greater advance (positive values) in their DLMO ($p < .05$, $R^2 = 0.36$).

Discussion

We used the timing of light interventions with the aim of modifying sleep timing and phase of entrainment. Our goal was to develop protocols to reduce SJL. Wearing blue-light-blocking glasses in the evening was effective in advancing both sleep timing (on workdays) and phase of entrainment (estimated via DLMO, measured only on workdays). In contrast, the intervention involving sleeping with open curtains did not significantly change the same parameters. In both conditions, a reduction of SJL was not observed. The most obvious explanation for this is that the significant changes in sleep timing were observed only on workdays (Study 1). With no appreciable change on work-free days, the SJL would actually increase rather than decrease.

While the negative effects of evening (blue) light exposure on sleep timing and alertness have been widely described (Chang, Aeschbach, Duffy, & Czeisler, 2015; Chellappa et al., 2013; Wahnschaffe et al., 2013; Wood, Rea, Plitnick, & Figueiro, 2013), little research has been done on interventions that decrease evening light exposure. Wearing blue-light-blocking glasses has been shown to significantly reduce both the suppression of melatonin by light and subjective alertness before bedtime (Sasseville, Paquet, Sevigny, & Hebert, 2006; van der Lely et al., 2015). To the best of our knowledge, this is the first study showing that wearing blue-light-blocking glasses in the evening is related to an advance in sleep onset and DLMO (on workdays). Both have large effect sizes.
Surprisingly, the effect was not maintained during the second intervention week. An adaptation to the new light regime and therefore a decrease in responsiveness to the intervention could explain the lack of a sustained effect related to wearing the orange glasses. Continuously wearing orange contact lenses for two weeks was found to decrease the sensitivity to light (reduced melatonin suppression; Giménez, Beersma, Bollen, van der Linden, & Gordijn, 2014). Our participants did not wear the blue-light-blocking glasses continuously, rather only in the evening. A study where participants wore glasses with yellow-tinted lenses for 8 hours a day found no evidence of adaptation in terms of color perception, suggesting that the sensitivity of the visual photoreceptors quickly renormalized once the glasses were removed (Tregillus, Werner, & Webster, 2016). Likely, the circadian system becomes less sensitive during reduced exposure to light but normalizes with increased light levels. We also cannot exclude a more trivial explanation for this finding, such as a reduction in compliance during the second intervention week. Although we have no indication for this, there was no sensor on the glasses to objectively measure when they were worn.

Study 2 did not show a significant change in sleep timing or in entrained phase following the light intervention (increased morning light). The beneficial effects of morning light in relation to sleep and depressive disorders have been described (Rosenthal et al., 1990; J. S. Terman, Terman, Lo, & Cooper, 2001), but there is a lack of (field) studies on the effects of morning light on the sleep-wake cycle in healthy individuals. A study run during winter in the Antarctic found that an hour of morning bright light advanced both sleep and melatonin rhythms (Corbett, Middleton, & Arendt, 2012). In our experiment, the light could not be described as ‘bright’; the participants who slept with open curtains were in fact not directly exposed to a light source. However, they received earlier light in their bedrooms during the intervention since twilight occurred earlier than their usual (baseline workdays) wake-up time (twilight time: 6:15 h; average wake-up time: 8:15 h). Similar to our findings, studies investigating the effects of artificial dawn on sleep have not found significant advances in sleep timing or DLMO (Giménez et al., 2010; Tonetti et al., 2015).

Additionally, there was great variation in the amount of increase of morning bedroom light during the intervention. Interestingly, the participants who experienced a larger increase in morning bedroom light levels experienced a larger advance in their DLMO. This significant correlation supports the idea that a stronger intervention would lead to advancing circadian phase. An alternative explanation is that there is heterogeneity in which individuals respond to light. This has been already described in some studies (Dijk et al., 2012; Santhi et al., 2011). Whether these individual differences in response to light are more pronounced in the morning compared to the evening needs further elucidation. Another point is that our study occurred in March when photoperiod changes rapidly (advance in dawn of 1 hour across the month) in The Netherlands. There is evidence that humans track dawn and advance their sleep timing especially in March (Kantermann, Juda, Merrow, & Roenneberg, 2007). This means that observing an additional advance in the intervention group could have been difficult. We also did not restrict our participants' behavior (e.g. light exposure in the evening), but it was shown that the advancing effects of morning light are counteracted by evening light exposure (Burgess, 2012).
Finally, we did not assess subjective parameters such as sleep inertia after waking in our study. Interestingly, Giménez and colleagues found that using a wake-up lamp (artificial dawn) decreased sleep inertia, which suggests that sleeping with open curtains could have the same beneficial effect (Giménez et al., 2010).

Based on the tools used in this study, the most effective way to advance sleep timing and phase of entrainment in late chronotypes would probably involve a combination of increased morning light exposure and decreased evening light exposure (Appleman et al., 2013). Alternatively, studies investigating individual differences in response to light at different times of day could determine whether for some individuals or chronotypes a light intervention in the morning is more effective than in the evening and whether for others the opposite is true.

Future studies should also investigate more long-term effects of such interventions to explore how the circadian system adapts to new light regimes and whether late chronotypes could find a stable, earlier phase of entrainment. Similar studies should be also repeated in participants suffering from extreme SJL to assess whether SJL can be decreased with light and whether this would lead to better health outcomes.

Acknowledgments

We thank Arjan Bos and Nienke Hoekstra for their help with the data collection and data analysis. Our work is supported by the Technology foundation STW grant P10-18/12186 and the University of Groningen.

Supplementary Information

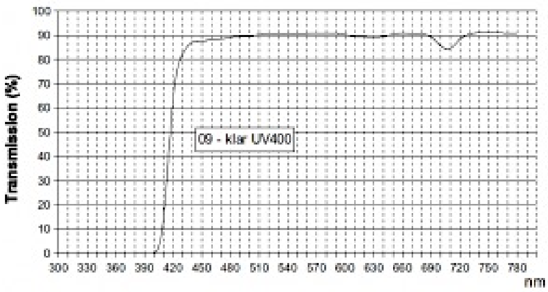

Figure S1. Transmission curve of the glasses with clear lenses

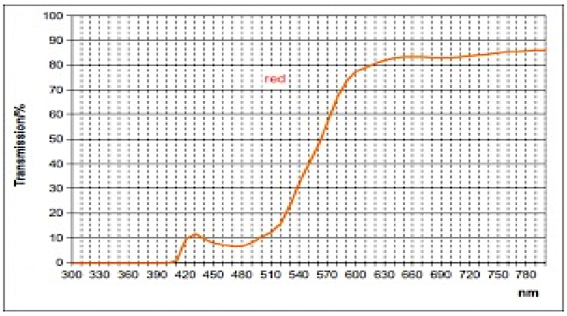

Figure S2. Transmission curve of the glasses with orange (blue-light-blocking) lenses

Chapter 7

Annual rhythms in school attendance and school performance

Giulia Zerbini, Vincent van der Vinne, Lana K.M. Otto, Till Roenneberg, Thomas Kantermann, and Martha Merrow

Abstract

The rotation of the earth around its axis and around the sun determines regular changes in the environment, such as the daily and seasonal alternation between day and night. While circadian rhythms have been extensively studied in humans, little is known about circannual rhythms. Examples of circannual variations have been described in birth and death rates, brain activation, sleep duration, and psychological state. The aim of this study was to investigate the impact of season on school attendance (late arrivals, dismissals from class, and sick leaves) and school performance (grades). We followed the same students over two consecutive school years (2013-2014 and 2014-2015). Students were asked to fill in the Munich ChronoType Questionnaire to assess chronotype, an estimation of individual phase of entrainment (synchronization). We found that school attendance varied according to time of year, with a peak in absenteeism in winter. Photoperiod (day length) was found to be the strongest predictor of school attendance. Early and late chronotypes did not differ in terms of seasonal variation of school attendance. In the school year 2013-2014, grades were highest in winter and lowest in summer. In the school year 2014-2015, grades were lower in fall compared to winter and summer. We would have expected grades to be lowest in winter (when absenteeism was highest) because our and previous studies showed a negative relationship between absenteeism and grades. However, it is possible that the effect of absenteeism on grades was delayed and not evident in winter. In addition, several other factors influence grades, decreasing the likelihood of detecting any seasonal variation in grades. This is supported by a different pattern in grades across seasons observed in the two academic years analyzed.

Based on these results, schools could start later in winter to increase school attendance, especially at high latitudes where there are substantial changes in photoperiod.

Introduction

The rotation of the earth around its axis determines the alternation between day and night in a 24-hour cycle. An internal time keeping mechanism – the circadian clock – gives the possibility to anticipate the regular changes in the environment rather than directly reacting to them. The clock regulates physiology and behavior creating a temporal structure that resonates with the 24h zeitgeber cycle. The earth's rotation changes the angular orientation relative to the sun over 365 days. This results in seasons with concurrent, systematic changes in temperature and day length (photoperiod).

While circadian rhythms have been extensively studied in humans, there are fewer studies on circannual rhythms. Most research about annual or seasonal biology concerns animals, where seasonality has economic consequences, e.g. fecundity (Paul, Zucker, & Schwartz, 2008; Rosa & Bryant, 2003). However, given that seasonality is so robust in many animals, it stands to reason that humans harbor some of this. Obviously, the amount of time required for this research limits the investigations in this field. In addition, with industrialization, the shift from outdoor to indoor living has reduced humans' exposure to seasonal signals such as photoperiod (day length) and temperature, possibly leading to a dampening of the seasonal variation in aspects of human life (Roenneberg, 2004). Studies investigating the seasonal variation in sleep behavior support this idea. In general, sleep is longer in winter than in summer, and this difference is much more pronounced in pre-industrial populations (Lehnkering & Siegmund, 2009; Okamoto-Mizuno & Tsuzuki, 2009; Yetish et al., 2015).

Other indications of seasonality have been described in some aspects of human life. Looking at birth records, Roenneberg and Aschoff found a seasonal pattern in European birth rates with a main peak in April/May and a second smaller peak in winter (Roenneberg & Aschoff, 1990). In North America and Eastern Europe the pattern was bimodal with a first peak in April and a second peak in November. The authors suggest that the seasonality in birth rates was probably a result of seasonality in conception (e.g. changes in hormonal levels). Likewise, sudden (cardiac) death has also been shown to vary according to time of year, with a peak in winter and a trough in summer (Arntz et al., 2000).
A recent study found evidence of seasonal variations in brain cognitive responses assessed with functional magnetic resonance imaging (Meyer et al., 2016). Interestingly the peaks and troughs in brain activation were shifted depending on the specific cognitive task that was assessed.
Finally, another example of seasonality in humans is the occurrence of depressed episodes in the seasonal affective disorder, being significantly higher in winter (Rosenthal et al., 1984).

Despite these studies, the function and the underlying mechanisms of circannual rhythms in humans are still poorly understood. For instance, the seasonal variation in sleep duration could be both related to changes in photoperiod and in temperature. Kantermann and colleagues (2007) found that sleep offset advances along with dawn from winter moving into spring, suggesting that humans are sensitive to changes in photoperiod (Kantermann, Juda, Merrow, & Roenneberg, 2007). Temperature (both internal and external) is also very much related to sleep, with especially the drop in core body temperature correlating with sleep onset (Kräuchi, 2007; Raymann, Swaab, & Van Someren, 2005). Both warmer and longer summer days could therefore hypothetically be signals to a circannual clock to modify sleep duration and timing according to season.

A better understanding of the role of individual differences in response to seasonality could increase our knowledge about the function of circannual rhythms. Individual differences in synchronization to the external light-dark cycle (chronotype) have been shown in humans (Roenneberg & Merrow, 2007). This is described as a distribution of chronotypes, ranging from early to late types (Roenneberg et al., 2007a). Chronotype is under the control of the circadian clock and can be modulated by light exposure (Roenneberg & Merrow, 2007). For these reasons, it could play a role in modulating responses to seasonal changes. Chronotype can be easily measured with questionnaires (Horne & Ostberg, 1976; Roenneberg, Wirz-Justice, & Merrow, 2003).

In the current study, we collected indicators of school attendance and school performance throughout two consecutive school years (2013-2014 and 2014-2015). This allowed us to assess annual rhythms in school attendance in the same students, and to compare the influence of chronotype, day length, and weather conditions on school attendance. We hypothesized that absenteeism would peak in winter, because sick leaves are more likely to occur during the colder months, and because sleep timing, especially in late chronotypes, is later in winter compared to summer (Allebrandt et al., 2014). Based on this, we also hypothesized that late chronotypes would be absent more often (particularly late more often) than early types in winter compared to summer. Finally, we expected grades to be worse in winter because of the negative effect of absenteeism on grades that we previously described (chapter 3). Access to this information will be important for understanding all of the forces at work that shape success and failure in school.

Methods

The study was performed at a Dutch high school in Coevorden (52° 40' N / 6° 45' E), The Netherlands, between August 2013 and June 2015. Data on late arrivals (during the first hour), dismissals from class (number of times a student was sent out from class by the teacher), sick leaves (number of times a student was on sick leave), and sick leave duration (duration of the sick leave in days) were retrieved from the school's registration system. The school day started at 8:15 h and ended at 15:45 h.

Between October and November 2013 a total number of 687 students filled in the Munich ChronoType Questionnaire (Roenneberg et al., 2003) to assess their chronotype (midpoint of sleep on school-free days corrected for sleep debt on school days; MSF_{sc}). Between August 2013 and June 2015, a total of 77,206 grades from examinations taken by students attending the first three school years (523 students during the school year 2013-2014 and 501 students during the school year 2014-2015; age range: 11-17 years) were collected. Of these students, 426 had filled in the MCTQ between October and November 2013.

Statistical analyses were done using R software version 3.3.0 (The R Core team, 2013) unless otherwise specified. The annual rhythm in weekly totals of late arrivals, dismissals from class, sick leaves, and sick leave duration was assessed using Circwave analysis in MS Excel (van der Veen, Mulder, Oster, Gerkema, & Hut, 2008). To investigate the effect of chronotype on the annual rhythm in school attendance, the students with known chronotype were divided into two equal sized groups of early ($MSF_{sc} \leq 4.31$) and late ($MSF_{sc} > 4.31$) chronotypes. Circwave analysis was repeated in these two groups separately and the resulting fits were compared. A stepwise backward regression analysis was performed to investigate the significance and strength of the unique contribution of several predictors to the yearly variance in school attendance. The factors analyzed in the model were weekly incidence of influenza registered in the Netherlands (per 100,000 inhabitants), weekly average day length (photoperiod in hours), and weekly average wind speed (in milliseconds), temperature (in degrees Celsius), and precipitations (in the hours from 6:00 h to 9:00 h, 1 hour time resolution, in millimeters) (sources: www.knmi.nl, Hoogeveen weather station: WMO #06279, 52° 43' N / 6° 28' E; http://ecdc.europa.eu/en/Pages/home.aspx).
Grades were collected during 4 periods (Fall: August - October; Winter: November - January; Spring: February - April; Summer: May - July). The annual rhythm in grades was assessed using a multilevel mixed model with grades as dependent variable and period of the year as independent variable. Student ID was analyzed as random factor nested within class and within level of education. Sex, school subject (geography, history, Dutch, English, biology, mathematics, chemistry, and physics), chronotype (MSF_{sc}), and school attendance variables were entered in the model as covariates. Age was not significantly associated with grades and therefore was not included in the model. Bonferroni correction was chosen for the post hoc tests.

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 Centre of Groningen (NL) and the head of the school approved the study.

Results

The number of students enrolled in the school years 2013-2014 and 2014-2015 was respectively 1,709 and 1,722. Data about chronotype and sleep timing were collected using the Munich ChronoType Questionnaire (MCTQ) in 687 students (350 females and 337 males, mean age 14.05 ± 1.63 SD; age range 11-18 years). Demographics and sleep timing data of these students are reported in Table 1. The number and percentage of students absent from class at least one time per school year in addition to the total number of late arrivals, dismissals from class, sick leaves, and total sick leave duration are reported in Table 2.

Table 1. Demographics of 350 female and 337 male students collected with the Munich ChronoType Questionnaire.

Outcome measure	Average (± SD)	Range
Age (years)	14.06 (1.63)	11-18
Chronotype (MSF_{sc}, h)	4.45 (1.15)	-0.38-10.58
Social Jetlag (h)	2.31 (1.02)	0.00-7.21
Sleep onset on school days (h)	-1.20 (0.99)	-3.42-4.00
Sleep end on school days (h)	6.67 (0.43)	4.5-8.25
Sleep duration on school days (h)	7.87 (1.03)	3.25-10.67
Sleep onset on school-free days (h)	0.34 (1.42)	-2.83-7.00
Sleep end on school-free days (h)	9.73 (1.50)	2.00-16.00
Sleep duration on school-free days (h)	9.40 (1.41)	1.5-13.98

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).

Table 2. Number and percentage of students absent from class (school years 1-6) and number and percentage of students with grades (school years 1-3).

	2013-2014		2014-2015	
Outcome measure	N students (%)	Count	N students (%)	Count
Late arrivals	735 (43%)	1,688	765 (44.4%)	1,911
Dismissals from class	502 (29.4%)	1,186	543 (31.5%)	1,382
Sick leaves	1300 (76.1%)	4,714	1347 (78.2%)	5,098
Sick leave duration (d)	1,300 (76.1%)	7,981	1,347 (78.2%)	8,872
Grades	523 (30.6%)	40,890	501 (29.1%)	36,316

The percentages are calculated relative to the total number of students enrolled that was 1,709 for the school year 2013-2014 and 1,722 for the school year 2014-2015. The total number (count in the table) of late arrivals, dismissals from class, sick leaves, days of sick leave, and grades is reported.

There was a significant annual rhythm in these parameters (Fig. 1A, late arrivals $F_{2,75} = 40.51$, $p < 0.0001$; Fig. 1B, dismissals from class $F_{2,74} = 6.183$, $p = 0.0033$; Fig. 1C, sick leaves $F_{2,75} = 49.50$, $p < 0.0001$; Fig. 1D, sick leave duration $F_{2,75} = 60.57$, $p < 0.0001$). All parameters peaked in winter, albeit each in a different week: late arrivals peaked during the first week of December; dismissals from class peaked during the last week of January; number of sick leaves and sick leave duration peaked during the second week of January.

To assess the influence of chronotype on school attendance in relation to time of year, the presence of an annual rhythm in school attendance was tested in two equal-sized groups of early ($MSF_{sc} \leq 4.31$) and late ($MSF_{sc} > 4.31$) chronotypes. Our results show that the annual rhythm in all indicators of school attendance was present in both groups. Visual inspection of the data did not suggest differences in phase or amplitude across the year between early and late chronotypes (Fig. 2). Late chronotypes were more often absent than early chronotypes independent of time of year (data in chapter 3).

To explore the observed annual rhythm in school attendance in more detail, the influence of several predictors that vary with time of year was assessed using backward stepwise regression (Fig. 3A-3D). Here we report the estimates (b coefficients) for the predictors present in the final model. The standardized coefficients (β) of each predictor in the initial and final model are reported in Table S1 (Supplementary Information). Day length and wind speed were the only factors significantly contributing to the variance in late arrivals (adjusted $R^2 = 0.53$; day length: $b = -6.861$, $t(75) = -9.425$, $p < 0.0001$; wind speed: $b = -4.775$, $t(75) = -3.059$, $p = 0.0031$). The model predicts that for each additional hour of daylight, there is a decrease by almost 7 late arrivals per week at the school level. Outdoor temperature was the only significant predictor of weekly number of dismissals from class ($b = -1.023$, $t(75) = -3.498$, $p = 0.0008$). The model predicts that if outside temperature increases by 1 °C, the weekly number of dismissals from class decreases by 1 unit at the school level. Both day length and outside temperature contributed significantly to the variance in number of sick leaves (adjusted $R^2 = 0.59$; day length: $b = -6.122$, $t(75) = -4.244$, $p < .0001$; temperature: $b = -3.583$, $t(75) = -4.001$, $p = 0.0001$), and to the variance in sick leave duration (adjusted $R^2 = 0.62$; day length: $b = -14.798$, $t(75) = -5.231$, $p < .0001$; temperature: $b = -6.375$; $t(75) = -3.629$, $p = 0.0005$). The model predicts that with each additional hour of daylight the number of sick students decreases by 6, and that the duration of the sick leaves decreases by 15 days at the school level. The model also predicts that when outside temperature increases of 1 °C the number of sick students decreases by 3, and the duration of sick leaves decreases by 6 days. The same analysis was repeated separately in early ($MSF_{sc} \leq 4.31$) and late ($MSF_{sc} > 4.31$) chronotypes to assess whether the predictors explaining the variance in school attendance were different depending on chronotype. The only difference between the two chronotype groups was found in relation to number of sick leaves. While in early chronotypes the only significant predictor was outside temperature (adjusted $R^2 = 0.32$, $b = -0.909$, $t(76) = -6.085$, $p < .0001$), in late chronotypes both outside temperature and day length contributed in explaining the variance in sick leaves (adjusted $R^2 = 0.37$; temperature: $b = -0.552$, $t(75) = 0.0228$; day length: $b = -1.153$, $t(75) = -3.013$, $p = 0.0035$). Day length was a stronger predictor than outside temperature for number of sick leaves in late chronotypes (day length: $\beta = -0.380$; temperature: $\beta = -0.293$).

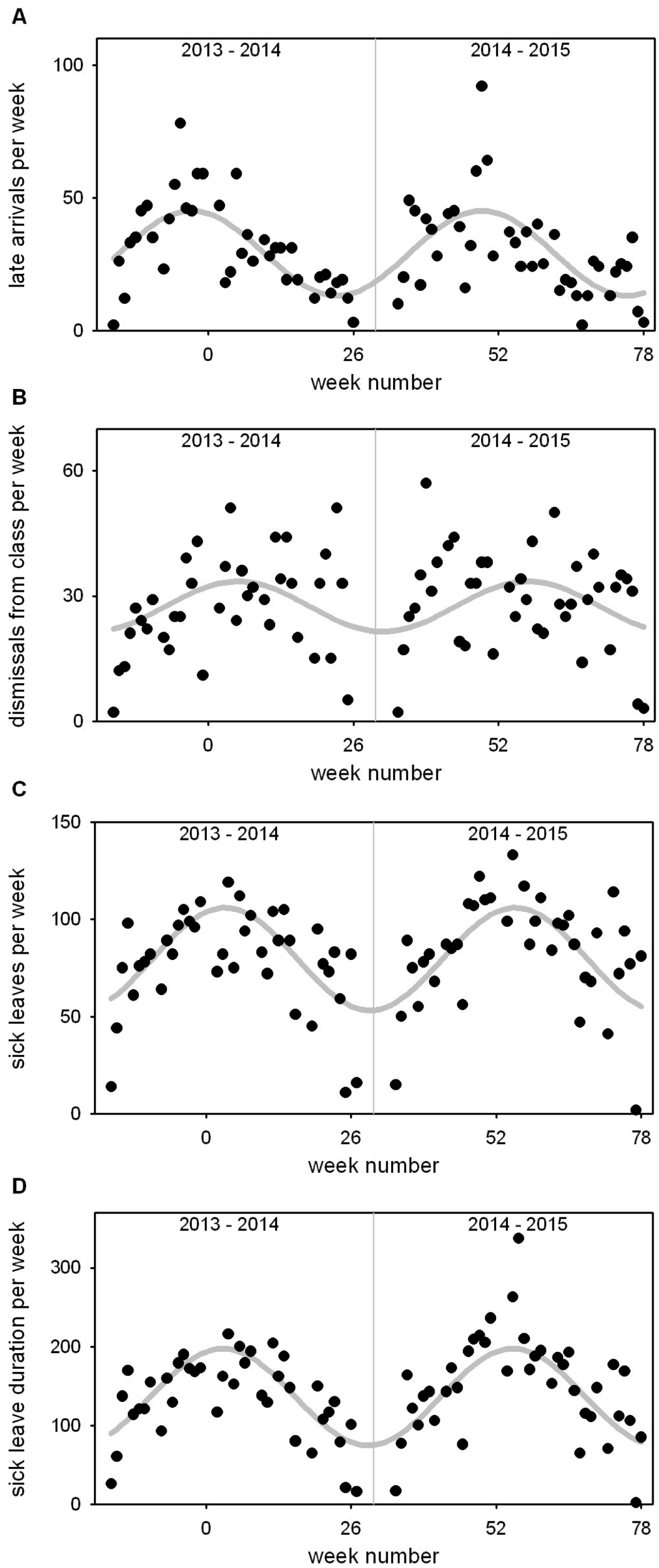

Figure 1. Annual rhythm in indicators of school attendance between September 2013 and June 2015. Week number 1 is the first week of January 2014. Curves represent the least-squares fits obtained using Circwave analysis. (A) The weekly number of late arrivals varied with time of year, with a peak in the first week of December. (B) The weekly number of dismissals from class varied with time of year, with a peak during the last week of January. (C-D) The weekly number of sick leaves and days missed due to sickness varied with time of year, with a peak during the second week of January.

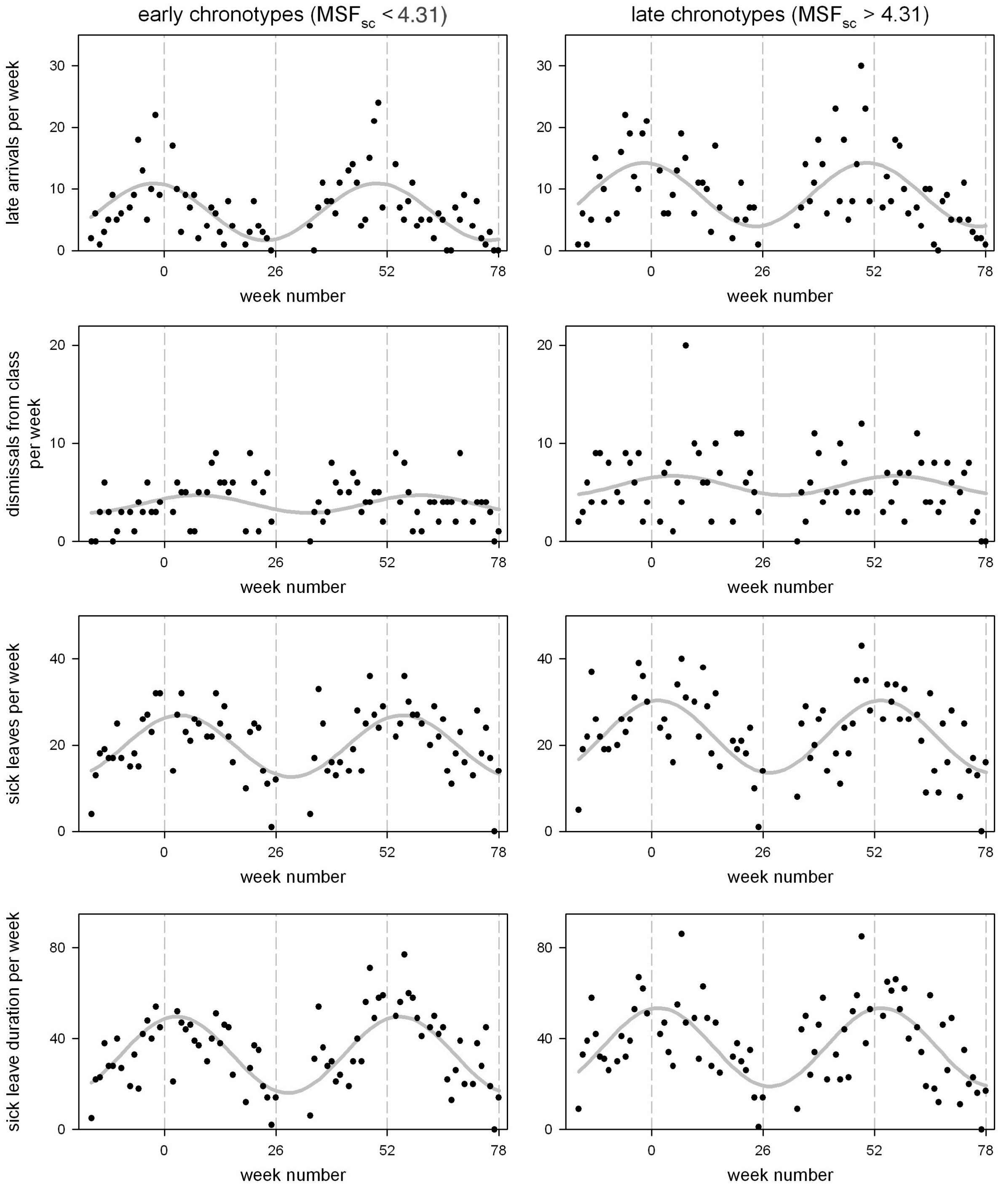

Figure 2. Annual rhythm in indicators of school attendance between September 2013 and June 2015 for early and late chronotypes.

Week number 1 is the first week of January 2014. Curves represent the least-squares fits obtained using Circwave analysis. The amplitude and peak phase of the yearly rhythm are equivalent for early and late chronotypes.

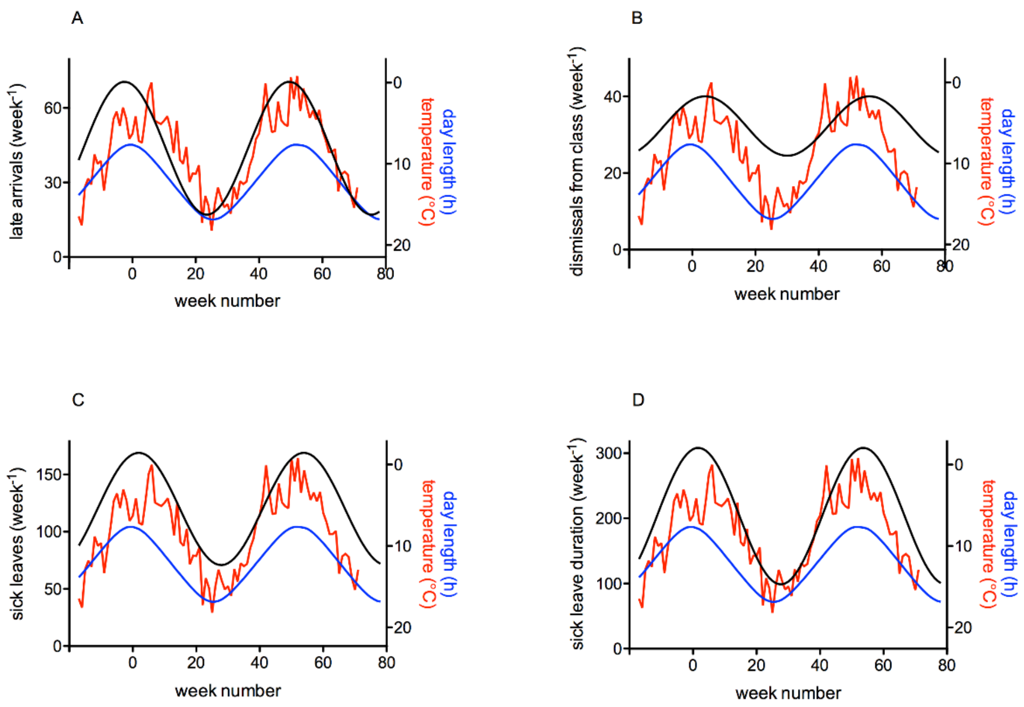

Figure 3. Annual rhythm in indicators of school attendance, temperature, and day length between September 2013 and June 2015.
Week number 1 is the first week of January 2014. Black curves represent the least-squares fits obtained using Circwave analysis for (A) late arrivals, (B) dismissals from class, (C) sick leaves, and (D) sick leave duration. In red and in blue the weekly values of outside temperature and of day length are plotted (reversed y axis).

The multilevel mixed model revealed that grades varied depending on season (Fig. 4A, 2013-2014: $F_{3, 32979} = 38.489$, $p < .0001$; Fig. 4B, 2014-2015: $F_{3, 21804.9} = 6.267$, $p = 0.0003$).
In the school year 2013-2014, grades were significantly different in each period, being best in winter and worst in summer. Compared to winter, grades were 0.06 units lower (on a scale from 1 to 10) in fall ($b = -0.059$, $t\ (32962) = -2.68$, $p = 0.042$), 0.14 units lower in spring ($b = -0.135$, $t\ (32958.5) = 6.21$, $p < .0001$), and 0.22 units lower in summer ($b = -0.217$, $t\ (32989.3) = 10.22$, $p < .0001$). In chapter 2 and 3 we described a chronotype-effect on grades, with late chronotypes obtaining lower grades compared to early chronotypes. To explore whether the strength and significance of this effect varied with time of year we added an interaction effect (chronotype x period) to the model and compared grades of early ($MSF_{sc} < 4.25$) and late chronotypes ($MSF_{sc} \geq 4.25$) in the four seasons. The interaction effect was significant ($F_{7, 3456} = 17.656$, $p <.0001$), showing that early chronotypes obtained better grades in summer compared to late chronotypes ($b = 0.2$, $t\ (625.5) = 2.78$, $p = 0.024$). In particular, grades of early chronotypes were on average 0.2 units higher (on a scale 1 to 10). During the other seasons, early chronotypes always obtained better grades compared to late chronotypes, but the differences were not significant (fall: $b = 0.2$, $t\ (690.1)$, $= 2.49$, $p = 0.052$; winter: $b = 0.1$, $t\ (627.5)$, $= 2.23$, $p = 0.104$; spring: $b = 0.1$, $t\ (656.5) = 2.2$, $p = 0.112$).

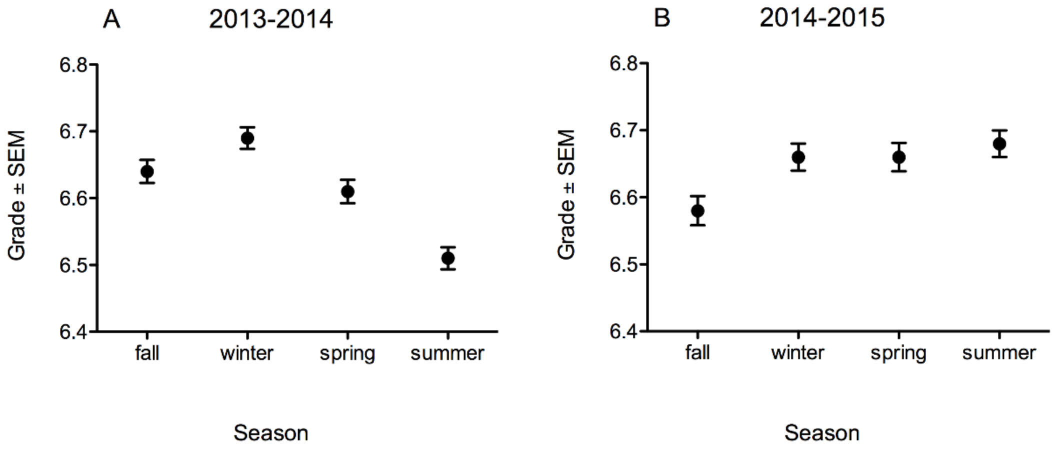

Figure 4. Annual rhythm in grades.
Data points represent mean grades with standard error of the mean (SEM). The grade averages were calculated on the row data per period (fall, winter, spring, and summer). In the school year 2013-2014 (A) grades were highest in winter and lowest in summer. In the school year 2014-2015 (B) grades were lower in fall compared to both winter and summer ($p < .05$ with Bonferroni correction).

In the school year 2014-2015, grades significantly differed only between fall and winter ($b = -0.078$, t (21803.5) = -2.74, $p = 0.036$) and between fall and summer ($b = -0.119$, t (21821.4) = -4.31, $p < .0001$). Compared to winter and summer, grades were respectively 0.08 and 0.12 units lower in fall. The interaction effect between chronotype and season was significant ($F_{7, 2436.5} = 3.013$, $p = 0.0037$), but the post hoc analysis did not reveal significant differences between early and late chronotypes over the four seasons (fall: $b = 0.1$, t (482.5), = 1.46, $p > .05$; winter: $b = 0.1$, t (450.1), = 0.96, $p > .05$; spring: $b = 0.1$, t (656.5) = 2.2, $p > .05$; summer: $b = 0.1$, t (424.6) = 1.17, $p > .05$).

Discussion

The main aim of the current study was to assess whether school attendance and school performance vary with time of year. Indicators for school attendance were collected between August 2013 and June 2015. We found an annual rhythm in school attendance with a winter peak in late arrivals, dismissals from class, sick leaves, and sick leave duration. Day length and weather conditions vary with time of year and may explain the observed variation in school attendance. The variation in day length in Coevorden (52° 40' N / 6° 45' E) is between 7:36 h (21st of December) and 16:53 h (21st of June), and the range in temperature between August 2013 and June 2015 in the hours from 6:00 h to 9:00 h (the time interval in which most students commute to school) was -4.7 °C and 24.8 °C, with the coldest temperatures between December and March. Winter has been associated with a peak in sick leaves because of a weaker antiviral response of the immune system at low temperatures (Foxman et al., 2015).

Likewise, the winter peak in late arrivals could have been explained by unfavorable weather conditions due to cold temperatures (e.g. presence of ice and snow on the streets). However, our results show that the strongest significant predictor for the annual rhythm in late arrivals was day length. Timing and duration of natural light exposure, together with other factors like genetic background, sex, and age, modulate an individual's phase of entrainment (chronotype) (Roenneberg et al., 2007a; Roenneberg & Merrow, 2007; Roenneberg et al., 2004). Thus sleep timing varies with the changes in day length across the year (especially at latitudes far from the equator), resulting in later and longer sleep in winter (Allebrandt et al., 2014; Kantermann et al., 2007). The number of late arrivals in our study also varied with day length, showing a peak in December when hours of daylight were lowest. If light exposure can influence school attendance through seasonal changes in phase of entrainment, we would expect to find less pronounced seasonal variation in school attendance in schools that are located closer to the equator (due to less annual rhythm in day length).

We did not observe differences in phase or amplitude of late arrivals depending on time of year between early and late chronotypes. Late chronotypes were throughout the year always more often late, but both early and late chronotypes showed the same annual rhythm in school attendance, with a peak in absenteeism in winter. During the winter months the students were exposed to less natural light: in December and January sunrise in Coevorden was later than the time at which the school started (mean sunrise time in December and January 08:35h, range 08:22h – 08:48h; school start time 08:15h). Indeed, exposure to a weak zeitgeber has been suggested to contribute to delayed sleep timing (Roenneberg, Kumar, & Merrow, 2007b). We did not assess sleep timing throughout the school year and, therefore, we can only speculate that students slept later and longer in winter compared to spring and summer, contributing to more late arrivals. When comparing early and late chronotypes, the same predictors for the different indicators of school attendance were found. The only difference between early and late chronotypes was related to sick leaves. In addition to temperature, day length was a significant predictor of sick leaves only in late chronotypes. It is possible that the sleep of late chronotypes is particularly influenced by the lack of winter morning light, leading to later sleep timing, more sleep deprivation and health related problems. To support this, increased exposure to natural light was shown to advance the melatonin phase more in late chronotypes than in early chronotypes, suggesting that changes in light exposure can have a bigger impact on sleep of late chronotypes (Wright et al., 2013).
The yearly variation in dismissals from class was less pronounced than that of late arrivals and sick leaves, and we do not have a clear explanation for this observation.

Finally, the seasonal variation in grades was different depending on school year. In 2013-2014, the average grade was different in each season, with the best grades obtained in winter and the worst in summer. In 2014-2015, grades were lower in fall compared to winter and summer. In contrast to the school attendance data, the grades were not collected on a weekly basis, but at the end of each period. This limited our seasonal variation analysis in that we could not try to fit a cosine wave through the data. Based on the observation of increased absenteeism in winter, we did not expect the grades to be highest at that time of year (2013-2014). However, it is possible that the negative effect of absenteeism on grades found in chapter 3 is delayed and becomes evident later in the school year via cumulative effects.

In addition, grades are influenced by many other factors, which also vary with time of year (for instance topics might be easier at the beginning of the school year), making any detection of a seasonal variation in grades depending on biological and environmental (e.g. photoperiod) factors very difficult.

Taken together, our novel finding that school attendance shows a significant annual rhythm with a peak in absenteeism during winter stimulates new ideas on how to increase school attendance and student performance. At high latitudes, for instance, schools could start later only in winter, reducing the higher rates of absenteeism during this time of year. In summer, an earlier school starting time could be kept, to increase morning light exposure in students and to also allow for after school – outdoor – activities.

Acknowledgments

We thank Jorrit Waslander and Hilde de Vries for their help in collecting the data. Our work is supported by the Technology foundation STW grant P10-18/12186 and the University of Groningen.

Supplementary Information

Table S1. Stepwise backward regression analysis of the influence of influenza incidence, day length, and weather conditions (i.e. wind speed, temperature, and precipitations) on the annual rhythm in school attendance.

	dependent variable	predictor	β	t-value	p-value
Model 1	Late arrivals	day length	-0.776	-6.829	< 0.0001
		wind speed	-0.220	-2.365	0.021
		temperature	-0.103	-0.804	0.424
		precipitation	-0.026	-0.288	0.774
		influenza	-0.141	-1.319	0.191
Model 2	Late arrivals	**day length**	-0.788	-9.425	**< 0.0001**
		wind speed	-0.256	-3.059	**0.003**
Model 1	Class dismissals	day length	-0.140	-0.751	0.455
		wind speed	0.004	0.028	0.978
		temperature	-0.303	-1.726	0.089
		precipitation	-0.148	-1.204	0.233
		influenza	0.016	0.108	0.914
Model 2	Class dismissals	**temperature**	-0.375	-3.498	**0.0008**
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Model 1	Sick leaves	day length	-0.417	-3.954	0.0002
		wind speed	-0.109	-1.260	0.212
		temperature	-0.388	-3.245	0.002
		precipitation	0.119	1.442	0.154
		influenza	0.075	0.758	0.451
Model 2	Sick leaves	**day length**	-0.432	-4.001	**< 0.0001**
		temperature	-0.408	-4.244	**0.0001**
Model 1	Sick leave duration	day length	-0.492	-4.938	< 0.0001
		wind speed	-0.098	-1.203	0.233
		temperature	-0.287	-2.543	0.013
		precipitation	0.110	1.411	0.162
		influenza	0.145	1.551	0.125
Model 2	Sick leave duration	**day length**	-0.509	-5.231	**< 0.0001**
		temperature	-0.353	-3.629	**0.001**

Standardized coefficients (β), t-values, and p-values for each predictor in the initial model (model 1) and in the final model (model 2) are presented. The p-value associated to the t-value for removal of a predictor was set at 0.05.

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, & Merrow, 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, & Merrow, 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, & Merrow, 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, Zvolsky, & 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 ± 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 ± 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).

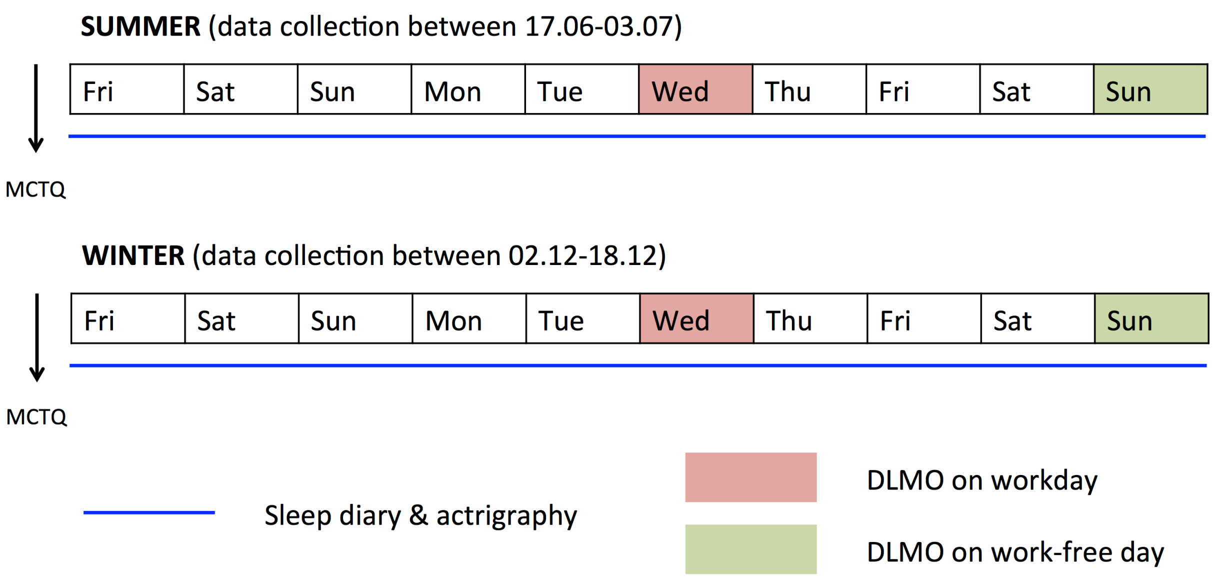

Figure 1. Experimental design.
Before each data collection, participants filled in the MCTQ. During the data collection, participants filled in a daily sleep diary and continuously wore an actiwatch (for light and activity data recording). On a workday (red) and on a work-free day (green), participants 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 (64^{th} 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 ± 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.

	Summer (17.06.16-03.07.16)		Winter (02.12.16-18.12.16)	
Parameter	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).

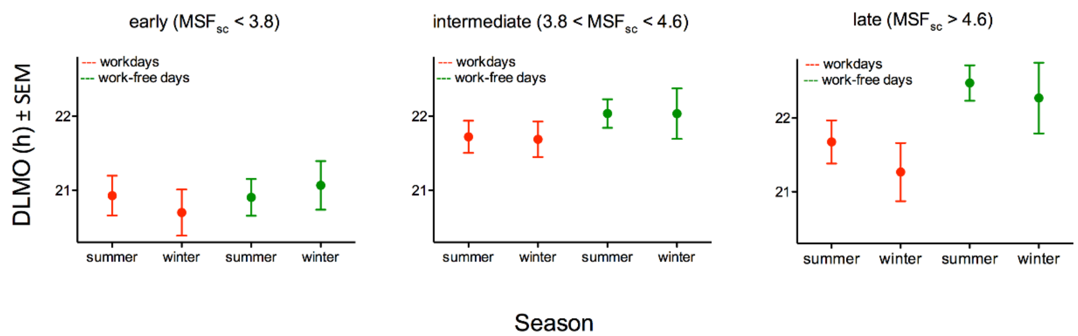

Figure 2. Chronotype-dependent variation in DLMO between workdays and work-free days. Data points represent means with standard error of the mean (SEM) of DLMOs assessed on workdays (in red) and on work-free days (in green) in summer and in winter. Data are plotted separately for early (MSF_{sc} < 3.8), 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.

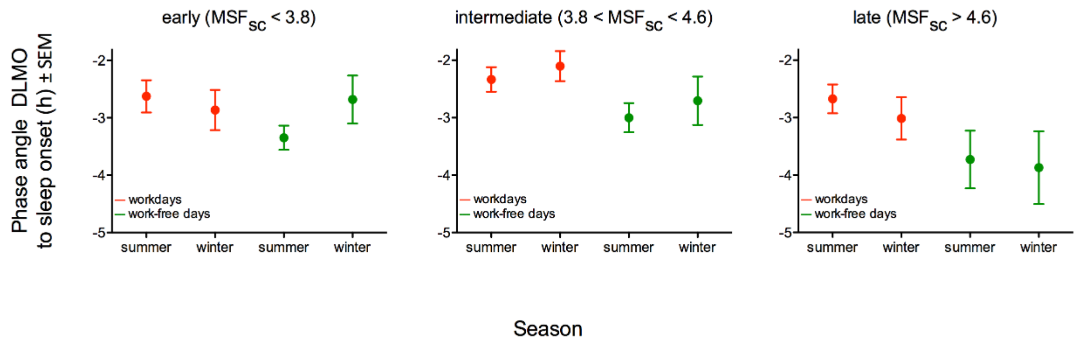

Figure 3. Chronotype-dependent variation in phase angle difference between DLMO and sleep onset on workdays and on work-free days.
Data points represent means with standard error of the mean (SEM). The phase angle difference between DLMO and sleep onset was calculated by subtracting sleep onset from 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.

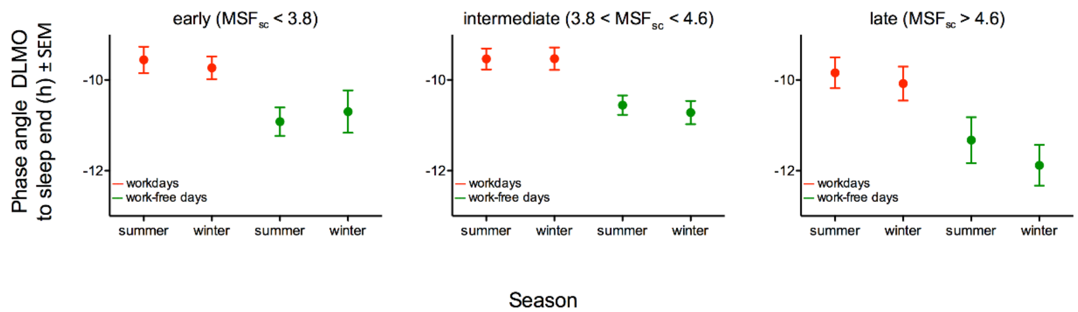

Figure 4. Chronotype-dependent variation in phase angle difference between DLMO and sleep end on workdays and on work-free days.
Data points represent means with standard error of the mean (SEM). The phase angle difference between DLMO and sleep end was calculated by subtracting sleep end from DLMO. 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 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.

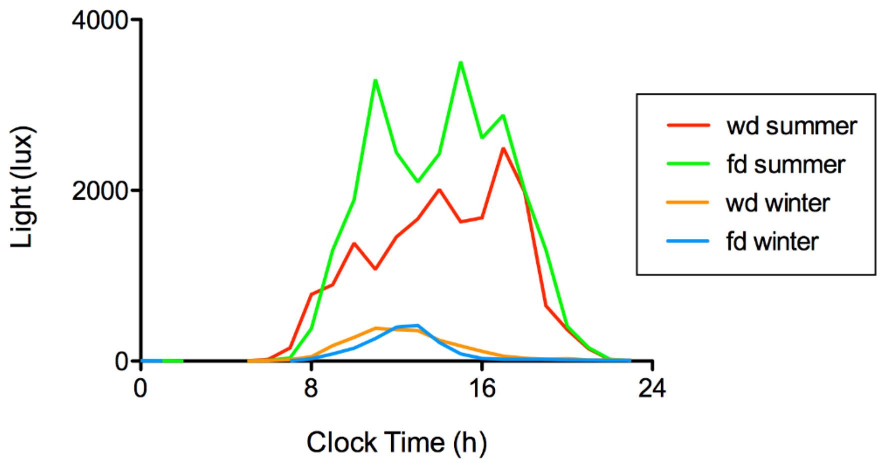

Figure 6. Light profile of all participants.
The average light intensities were calculated per hourly bins separately for workdays in summer (red), work-free days in summer (green), workdays in winter (orange), and work-free days in winter (blue). In summer participants were exposed to more light. On 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.

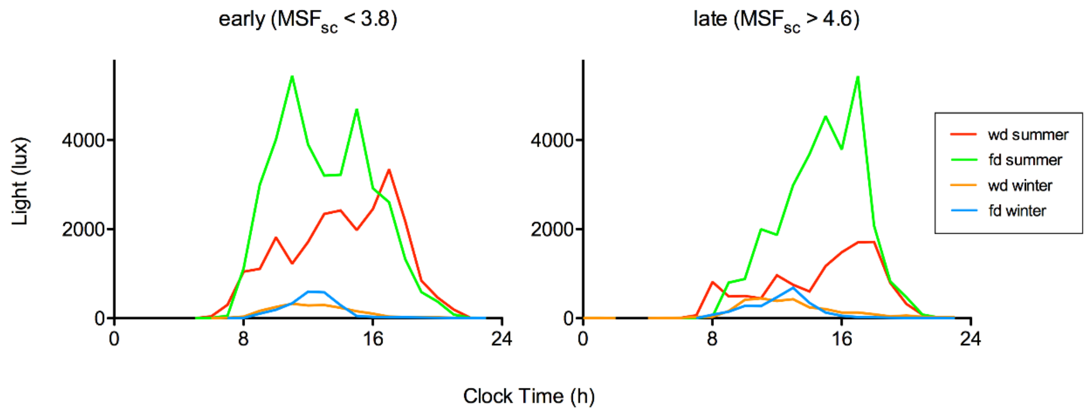

Figure 7. Light profile of early and late chronotypes.
The average light intensities were calculated per hourly bins separately for workdays in summer (red), work-free days in summer (green), workdays in winter (orange), and work-free days in winter (blue). The model indicates that late chronotypes were 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 & Merrow, 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).

Chapter 9

Conclusion

Giulia Zerbini

The conflict between internal and external time signals challenges the entrainment of the circadian clock. Internal time, sun time and social time are often not perfectly synchronized. In addition, although internal time varies substantially between individuals (chronotypes), everyone is expected at work (and at school) at the same social time. This thesis had three main objectives: 1) to describe the consequences of conflicting clocks; 2) to test solutions to decrease the mismatch between the circadian and the social clocks; 3) to better understand entrainment in real life conditions. In the following paragraphs, the main results of this thesis will be discussed, with a particular focus on the applicability of the findings and with an outlook to new hypotheses that were generated with this work and that could be tested in future studies.

Part 1 - Conflicting clocks: chronotype and school (academic) performance

Main results

In chapter 2 and 3, we studied the role of chronotype together with time of day and school attendance in relation to school performance (grades). Previous literature had shown that late chronotypes usually obtain lower grades compared to early chronotypes (Borisenkov, Perminova, & Kosova, 2010; Escribano, Díaz-Morales, Delgado, & Collado, 2012; Randler & Frech, 2009; van der Vinne et al., 2015; Vollmer, Pötsch, & Randler, 2013). We showed that the chronotype-effect on grades was modulated by time of day, with late chronotypes underperforming early chronotypes in the morning but not in the early afternoon (van der Vinne et al., 2015). In addition, we found that the chronotype-effect on grades was stronger for scientific subjects. Chronotype also influenced school attendance, with late chronotypes being more often absent, and absenteeism, in turn, was also associated with lower school performance.

In chapter 4 we aimed to expand our previous results concerning the interaction effect between chronotype and time of day on grades. For this purpose, we chose to assess the academic performance of university students because their examination schedules ranged from early in the morning to late in the evening. Unfortunately, the number of grades collected in the evening was much lower relative to the grades collected in the morning and afternoon, thus limiting the interpretation of our findings. Interestingly, in this study chronotype was found to be associated with attendance as well, with late chronotypes attending fewer lectures. In addition, lecture attendance and study effort were found to be more strongly associated with academic performance than chronotype.

Discussion points

The influence of chronotype on performance in high-school vs. university students

Based on our studies, the effect of chronotype on school performance in high-school students cannot be generalized to university students. Chronotype was in fact a significant predictor of grades in high-school students, but not in university students. In the latter case, factors such as lecture attendance and study effort seemed more important for academic success. This observation is supported by a recent meta-analysis, showing that the strength of the chronotype-effect on grades is greater in studies with high-school students rather than university students (Tonetti, Natale, & Randler, 2015). Our finding that the chronotype-effect on grades is modulated by time of day, being stronger in the morning and disappearing in the afternoon, could explain this difference between high-school and university students. High-school students have in fact usually a more regular schedule (often starting early in the morning), while university students have more flexibility and can sometimes also choose not to attend the lectures. There is another possible explanation for a weaker effect of chronotype on grades in university students when considering in particular the Dutch education system. Approximately at the age of 11, students are already selected to attend different levels of education based on their grades. The different levels of education determine the future opportunities in a student's academic career (i.e. possibility to apply for technical or research universities). Since early chronotypes obtain better grades and students with better grades can apply to research universities, it is possible that there is a higher prevalence of early chronotypes (relative to that age), reducing therefore the effect of chronotype on grades.

Lower school performance in late chronotypes: possible mechanisms

Our findings, as well as those from others, suggest a complex interaction between chronotype and other factors important for school performance. In general, chronotype seems to have both a direct and an indirect effect on school performance. The indirect effect is mediated by other factors, such as conscientiousness or motivation. For instance, chronotype was found to influence conscientiousness, with early chronotypes scoring higher on this personality factor, and students with higher conscientiousness, in turn, obtaining better grades (Arbabi, Vollmer, Dörfler, & Randler, 2014; Rahafar, Maghsudloo, Farhangnia, Vollmer, & Randler, 2016). The relationship between chronotype and sleep duration on school days deserves some attention as well, since several reviews have reported an association between short sleep duration and lower school performance (Curcio, Ferrara, & De Gennaro, 2006; Dewald, Meijer, Oort, Kerkhof, & Bögels, 2010; Taras & Potts-Datema, 2005; Wolfson & Carskadon, 2003). Late chronotypes usually sleep shorter on school/working days (Roenneberg et al., 2007), and, therefore, the effect of chronotype on school performance could be the result of being tested at a non-optimal time of day or of being sleep deprived or both. It is very difficult to disentangle these effects. We statistically attempted this by performing a model selection on a different set of predictors. The model with chronotype, and not that with sleep duration (on school days), was selected as the model with the most parsimonious fit to explain the variation in school grades. When both chronotype and sleep duration were in the same model, only chronotype was significantly associated with grades. These statistical analyses suggest that the isolated effect of chronotype has a larger impact on grades than the isolated effect of sleep duration. In addition, data from chapter 2 (not previously discussed) show that Tuesday was the weekday with the lowest grades (Fig. 1). If sleep duration were the most important factor for school performance and students were accumulating a sleep debt across the week, we would expect a progressive decline in school performance with the lowest grades obtained on Friday (end of the week). This would be a typical effect of chronic sleep restriction on performance described in previous studies (Dinges, Pack, Williams, & Gillen, 1997; Van Dongen, Maislin, Mullington, & Dinges, 2003). In contrast, the lowest grades were obtained at the beginning of the week (especially on Tuesday). Another phenomenon related to chronotype, namely social jetlag, could explain these results. On the weekend, adolescents usually sleep later and longer, which results also in a delay of their dim-light melatonin onset (Crowley & Carskadon, 2010). It is therefore possible that students are tested at an earlier internal time at the beginning of the week (relative to later in the week) because of the delay in phase of entrainment over the weekend. We showed in chapter 2 that when students are tested too early in their internal day, grades are significantly lower. This could therefore explain the lowest school performance of students at the beginning of the week.

Finally, it is important to mention that we did not collect any information about napping behavior in the students. It is possible that the negative effects of short sleep duration on grades were not evident because students compensated with naps for the daily sleep debt.

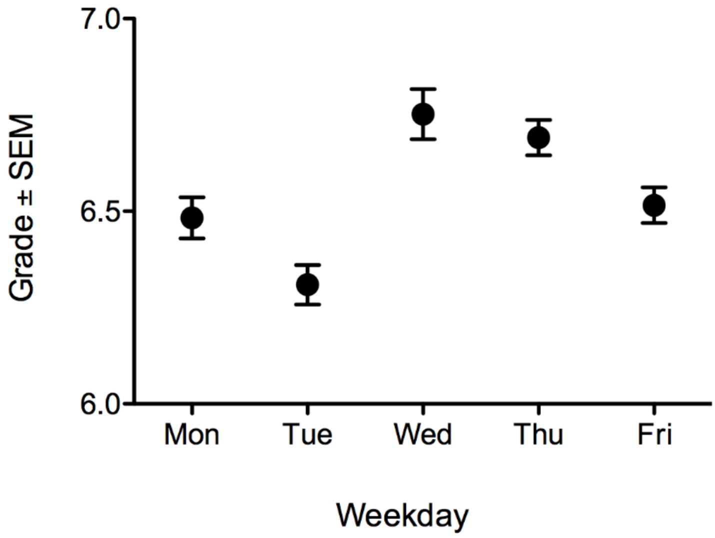

Figure 1. Grades by weekday.
There was a significant main effect of weekday on grades ($F_{4,3848}$ = 16.833, p < .0001). Post hoc test with Bonferroni correction for multiple comparisons showed that grades on Tuesday were significantly lower compared to grades on any other day of the week. In addition, grades on Friday were lower compared to grades on Thursday.

This thesis added two main novel results to the growing literature about the relationship between chronotype and school performance: the dependency on time of day and on school subject of the chronotype-effect on grades. Both findings give rise to new interesting questions and hypotheses. For instance, based on the results from chapter 2 and on the literature about the "synchrony effect" (May, Hasher, & Stoltzfus, 1993), we hypothesized that late chronotypes would obtain higher grades compared to early chronotypes if tested in the evening. Unfortunately, we could not test this hypothesis in high-school students (no examinations later than 16:00 h). University students did take examinations in the evening (18:00 h), but we were not able to collect enough data at that particular time of day.

Therefore, whether late chronotypes would take advantage from examinations scheduled in the evening is still an open question that could be answered in future studies.
Our finding that the chronotype-effect on grades is stronger for scientific subjects suggests that chronotype might influence specific cognitive abilities. Fluid intelligence (abstract thinking, logic, reasoning) is thought to be more relevant for scientific rather than humanistic/linguistic subjects (Chapelle & Green, 1992; Primi, Ferrão, & Almeida, 2010). Several studies have found a chronotype-effect on cognitive tasks requiring fluid intelligence but not crystallized intelligence (general knowledge) (Fimm, Brand, & Spijkers, 2015; Goldstein, Hahn, Hasher, Wiprzycka, & Zelazo, 2007; Lara, Madrid, & Correa, 2014). Therefore, all these studies support our hypothesis that a lower school performance in late chronotypes is related to deficits in fluid cognition. More studies are needed to determine at which level of cognition chronotype has an impact. For instance, being tested at a non-optimal time of day (and in deficiency of sleep) could slow down cognitive speed, with this being mainly reflected in fluid intelligence rather than crystallized intelligence.

Importantly, these results can be used to develop new school policies. For instance, examinations could be scheduled later in the day especially for scientific subjects. Another way to improve school performance in late chronotypes would be delaying school starting times. The main argument in favor of delaying school starting times is that the current school system discriminates students based on chronotype, a biological trait that shows an extreme inter-individual variability (Roenneberg et al., 2007). In addition, since chronotype delays during adolescence, the majority of students sleeps too little on school nights and would probably benefit from later school starting times (C. E. Basch, Basch, Ruggles, & Rajan, 2014; Crowley et al., 2014; Roenneberg et al., 2004). In Figure 2 the correlation between chronotype and sleep duration on school days in 741 students (data from chapter 2) is plotted. It is clear that not only late chronotypes, but rather almost all students (89%) do not get the recommended 9 hours of sleep for adolescents (Carskadon, 1990). If school starting times were delayed by half an hour (from 8:15 h to 8:45 h) and sleep onset remained stable, the percentage of students getting at least 9 hours of sleep per school night would increase from 11% to 26%. Delaying the school starting times by 1 hour would allow almost half of the students (46%) to sleep 9 hours. The main argument against delaying school starting times concerns the risk that adolescents would just delay their sleep even more. A recent study used mathematical modeling to make predictions on how changes in social schedule vs. changes in evening light exposure would affect phase of entrainment (Skeldon, Phillips, & Dijk, 2017). According to the model, delays in social schedules are beneficial only when the social schedules originally started before dawn. Otherwise, there is a risk that a delay in social schedules could translate into a delay in phase of entrainment. In addition, controlling evening light exposure seems more effective in modifying phase of entrainment and decrease, for instance, social jetlag, than changing social schedules. In contrast to this view, several studies have already shown the beneficial effects of delayed school starting times on attendance, performance, sleep, and health (Boergers, Gable, & Owens, 2014; Carrell, Maghakian, & West, 2011; Owens, Belon, & Moss, 2010; Owens, Drobnich, Baylor, & Lewin, 2014; Wahlstrom et al., 2014). However, the long-term effects of delaying school starting times still need to be clarified.

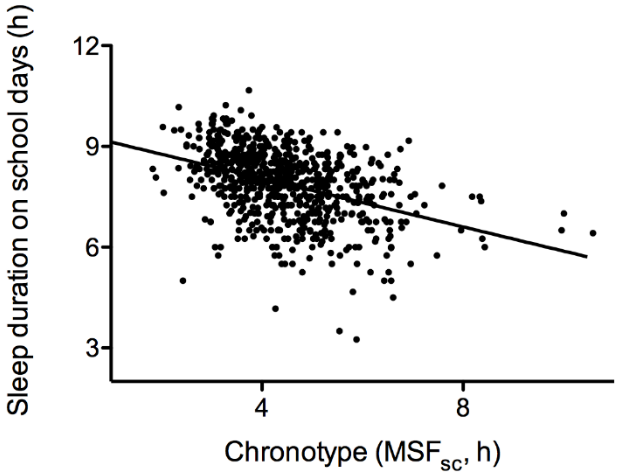

Figure 2. Sleep duration on school days in high-school students with different chronotypes.
Late chronotypes sleep shorter on school days (R^2 = .17, p < .0001). In addition, 89% of the students (N=741) sleep shorter than 9 hours.

We also planned to run a study to investigate the effects of later school starting times on school attendance and performance in a high school in The Netherlands. Unfortunately, the school not only delayed school starting times by 25 minutes (from 8:05 h to 8:30 h), but also introduced a new organization of the school day, making it difficult to disentangle the effects of these two changes on school attendance and performance.

Finally, the seasonal variation in school attendance reported in chapter 7 suggests additional, interesting solutions to improve attendance and performance in especially late chronotypes. Since absenteeism was found to be highest in winter (in The Netherlands where seasonal changes are substantial), seasonal opening times could be implemented, with later school starting times only in winter to increase attendance.

Taken together, as suggested in chapter 5, the optimal solution for improving school performance in late chronotypes involves probably a delay in school starting times associated with interventions (e.g. decreased evening light exposure) to ensure that adolescents do not further delay their phase of entrainment.

Part 2 - Conflicting clocks: light interventions to decrease social jetlag

Main results

In chapter 6 we tested the effectiveness of two light interventions to decrease the mismatch between the circadian and social clocks (social jetlag). We found that sleeping with bedroom curtains open (increased morning light exposure) did not significantly advance sleep timing and phase of entrainment (assessed via dim-light melatonin onset; DLMO) at the group level.

Still, we found a correlation between the shift in DLMO and the amount of increased light in the bedrooms when sleeping with open curtains: DLMO advanced more in those participants who had a greater increase of morning light intensity in their bedroom during the intervention. In the study involving a decrease in (blue) light evening exposure we found that both sleep timing on workdays and DLMO significantly advanced at the group level during the first intervention week.

Discussion points

Decreasing social jetlag with light: is it possible?

In both studies, we did not observe a decrease in social jetlag because sleep timing on work-free days did not significantly change. However, the way social jetlag is assessed (absolute difference between midpoint of sleep on workdays and on work-free days) might not detect a reduction of the mismatch between the circadian and the social clocks. In fact, an advance in sleep timing on workdays means that late chronotypes were actually sleeping more in synchrony with their social clock (and less with their circadian one) during the working week.

It is also important to mention that in both studies social jetlag at baseline was low (on average less than 2 hours). Similarly, sleep duration on workdays was not extremely short (on average 7.5 hours). This means that there was not much room for improvement via our interventions. The reason for these "good" baseline values (social jetlag and sleep duration on workdays) probably derives from the average Dutch working hours (9:00 h to 17:00 h) that do not challenge too much the sleep of late chronotypes. Students start school, in contrast, between 8:00 and 9:00, depending on the school.

Taken together, these experiments should be repeated in extreme late chronotypes suffering from more than 2 hours of social jetlag. In such a population, the light interventions advancing phase of entrainment should lead to longer sleep duration on workdays, less oversleep on work-free days, resulting in a decrease in social jetlag as we hypothesized.

Advancing phase of entrainment: more morning light vs. less evening light

Based on the results in chapter 6, decreasing evening (blue) light seemed a more effective intervention to advance sleep and phase of entrainment (on workdays). However, the correlation found between the advance in DLMO and the increase in bedroom light (when participants were sleeping with curtains open) suggests that our morning light intervention was not strong enough. Indeed, we observed a great variation in bedroom light intensities between individuals. Several factors such as size and orientation (e.g. east, north) of the windows could explain these differences. In addition, participants were directly exposed to more morning light only if they were first woken up by the light. With eyelids closed, in fact, the amount of light reaching the retina is reduced by 97 % especially for wavelengths lower than 590 nm, that are the most important for resetting the circadian clock (Brainard et al., 2001; Provencio, Jiang, De Grip, Hayes, & Rollag, 1998; Robinson, Bayliss, & Fielder, 1991).

The curtains experiment could be repeated adding a positive control group that wears blue-light-emitting glasses in the morning. In addition, a follow-up study testing the two interventions (more morning light exposure and less evening light exposure) in the same participants in a crossover design could clarify which intervention is more effective to advance phase of entrainment. For applicability, such studies should also have the aim of determining how long someone should be exposed/shielded from light to achieve the desired shift in phase of entrainment. For instance, if wearing blue-light-blocking glasses in the evening for about 3-4 hours can advance DLMO by 30 minutes and the same is achieved by being exposed to blue light in the morning for 1 hour, the latter intervention would be probably preferable.

The role of individual differences

When considering possible applications of these findings, it is also important to educate people about the counteracting effects that their behavior could have on the light intervention. For example, if the intervention is in the morning but the evening light exposure is still considerable, the intervention might not be effective (Burgess, 2012). Indeed, different behaviors of the participants could explain individual differences in responding to light. The inter-individual variability in response to light is substantial, often described, but poorly understood (Dijk et al., 2012; Santhi et al., 2011). Does sensitivity to light change across individuals and with time of day? Is this dependent on chronotype? Can these individual differences be explained due to the fact that interventions are timed in reference to external time rather than internal time? Early and late chronotypes exposed to light at the same external time could respond with a phase delay or a phase advance since their internal time may be extremely different. The classical phase response curve (PRC) to a light stimulus shows how the circadian clock responds to light presented at different times of day and is usually done with intermediate types. Would the PRC of an early type differ from the one of a late type? More studies are needed to understand individual differences with the final aim of tailoring interventions to the specific characteristics and needs of the individuals. For instance, interventions should be timed based on internal time and not external time (as we did in the orange glasses study),

Light interventions: short-term vs. long-term effects

Another important aspect to consider is the duration of the effects of the light intervention. For instance, we found that wearing the blue-light-blocking glasses significantly advanced sleep and phase of entrainment only during the first intervention week. Several explanations are possible for these non-lasting effects. The most trivial one is a lack of compliance during the second intervention week. We have no reason to hypothesize this, but we also did not have any objective measure (e.g. motion sensor on the glasses) to control for compliance. Another explanation could be that the participants adapted to the new light regime and therefore the intervention was not effective anymore during the second intervention week. However, the participants did not wear the glasses continuously and were therefore exposed to light during the day, which probably restored their baseline sensitivity to light every day.

Finally, it is possible that the timing of the intervention (fixed for both weeks) was not the same in terms of internal time between the two weeks. If we consider that the participants advanced their phase of entrainment (DLMO) after the first intervention week, they probably started wearing the blue-light-blocking glasses at a later internal time during the second week. This, in turn, could have exposed part of their delaying portion (PRC) to light (Fig. 3).

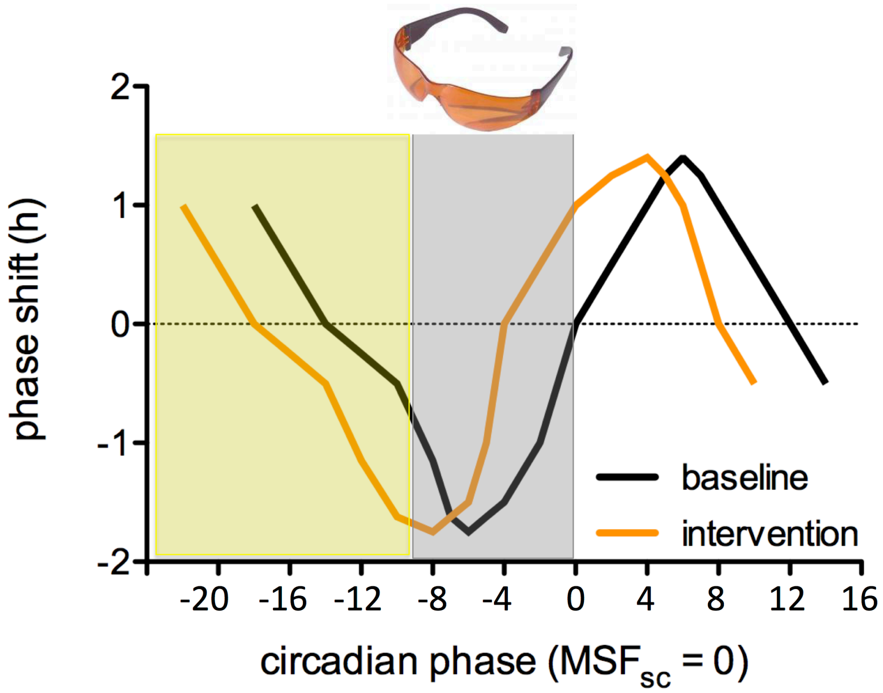

Figure 3. Possible mechanisms for the short-term effect of less evening light.
The typical phase response curve (PRC) to a light stimulus presented at different times of day is plotted. When exposed to light during the early biological night (here during the hours before MSF_{sc}), the circadian clock responds with phase delays (negative numbers on the y-axis). When exposed to light during the late biological night (here during the hours after MSF_{sc}), the circadian clock responds with phase advances (positive numbers on the y-axis). The intervention (wearing orange glasses; grey area) was individually timed (9 hours before MSF_{sc}) to reduce light exposure during the delaying portion of the PRC. The timing when participants had to wear the glasses was fixed for both intervention weeks. However, if phase of entrainment of the participants advanced already after the first intervention week (orange curve), it is possible that some of the delaying portion was exposed to light (yellow area) during the second intervention week. This could explain a reduced response to the intervention during the second week.

Part 3 - Understanding entrainment in real life conditions

Main results

In chapter 7 and 8, we aimed to better understand entrainment in real life conditions by assessing the influence of season and weekly schedule on behavior (school attendance and performance), sleep, activity, and phase of entrainment (DLMO).

The analysis of two consecutive years of data (chapter 7) revealed an annual rhythm in school attendance (late arrivals, dismissals from class, sick leaves). Absenteeism was found to be highest in winter. Among the several predictors of school attendance analyzed, photoperiod (day length) was the strongest (especially in relation to late arrivals).

In chapter 8, we aimed to better understand the influence of season and weekly schedule (workdays opposed to work-free days) on sleep timing, phase of entrainment (DLMO), the relationship between these two parameters, and sleep/wake timing. In addition, we assessed chronotype to investigate whether the influence of season and weekly schedule on these variables varied with chronotype. Activity, sleep, DLMO, and the phase relationship between sleep and DLMO did not vary with season. This was somehow surprising since light intensities (most important zeitgeber for human entrainment) were much higher and light exposure was longer in summer compared to winter. Morning light (which was higher in summer) was also the strongest predictor for the variation in DLMO. In contrast, weekly schedule influenced all the variables assessed. DLMO was earlier on workdays compared to work-free days both in summer and in winter. The difference in DLMO between workdays and work-free days was more pronounced in the latest chronotypes (not significant in early chronotypes). Late chronotypes were also exposed to less and later morning light on work-free days, possibly explaining the delay in DLMO over the weekend. Similarly, both sleep and activity were earlier on workdays. The phase angle difference between DLMO and sleep was smaller on workdays.

Discussion points

Seasonal variation in school attendance

In chapter 7, we did not assess sleep throughout the year and therefore we can only advance hypotheses to explain the influence (direct or indirect) of photoperiod on school attendance. We first hypothesized that sleep was later in winter increasing the chances of oversleeping and arriving late at school. We based this hypothesis on a previous study showing that sleep (especially in late chronotypes) was later in winter (Allebrandt et al., 2014). However, this was a cross-sectional study and we showed in chapter 8 that sleep timing assessed in the same individuals (working population) did not change between summer and winter. Another hypothesis could be that sleep inertia is longer in winter. Although students might have slept at the same time, getting up when it was still dark (winter) could have been more difficult, leading to more late arrivals. This hypothesis is supported by a study showing that waking up with a wake-up light decreased sleep inertia (Giménez et al., 2010). To test these hypotheses and to confirm the role of photoperiod, future studies could collect data about school attendance, sleep, and sleep inertia across seasons in a school at high latitude and in a school at latitude closer to the equator. We expect the annual rhythm in school attendance to be reduced or even to disappear in a school at latitude close to the equator where the changes in photoperiod between seasons are very small.

Seasonal variation in light exposure but not in phase of entrainment

The absence of a change in sleep, activity, and DLMO between summer and winter was quite unexpected, since the importance of light in human entrainment is known, and the variation in light exposure between the two seasons was substantial. The contrast between day and night is greater in summer and this should influence phase of entrainment. However, it is possible that the circadian system adapts its response to light to the gradual changes in light intensities in order to keep a stable phase of entrainment across seasons. In addition, not only the average light intensities vary with season but also the duration of light exposure and when light is available. Namely, the expansion in photoperiod during summer is symmetrical, increasing light exposure in the morning but also in the evening. Since light exposure at these two times of day has opposite effects on our phase of entrainment, it is possible that, as a consequence, phase of entrainment does not change between summer and winter. This would depend on balanced exposure (with respect to phase changing potential) at the beginning and at the end of the day.

Two general limitations are worth mentioning about studies investigating the influence of season on human entrainment. First, DLMO might not be the best marker to assess seasonal variations in entrainment, since a longer secretion of melatonin in winter (long photoperiod) has been reported (Stothard et al., 2017; Wehr, 1991), and this could explain an advance in DLMO in winter (relative to summer). Second, most countries in the world adopt daylight saving times (DST) during the summer months. The consequence is a delay by 1 hour of social time between April and October. This has been shown to disrupt entrainment in humans (Kantermann, Juda, Merrow, & Roenneberg, 2007), and raises the question whether the variables assessed should be corrected or not for DST.

The influence of the weekly schedule on phase of entrainment

We reported here for the first time a chronotype-dependent delay in DLMO on work-free days compared to workdays in a working population that received no restriction to their habitual behavior. We linked this difference in DLMO between workdays and work-free days to a later and decreased morning light exposure observed only in late chronotypes. Our results are supported by and bring together several studies that have shown 1) earlier light exposure on workdays compared to work-free days (Crowley, Molina, & Burgess, 2015); 2) earlier light exposure in early chronotypes compared to late chronotypes (Goulet, Mongrain, Desrosiers, Paquet, & Dumont, 2007); 3) delay in DLMO after sleep timing and/or duration had been manipulated to simulate a typical weekend (Burgess & Eastman, 2006; Crowley & Carskadon, 2010; Jelínková-Vondrasová, Hájek, & Illnerová, 1999; Taylor, Wright, & Lack, 2008; Yang, Spielman, & Ambrosio, 2001).

This novel finding needs to be replicated in future studies since it challenges two important concepts in chronobiology: the concept of social jetlag (Fig. 4) and of DLMO as phase marker of the circadian clock. Originally, social jetlag was described as a mismatch between the circadian and the social clocks (Wittmann, Dinich, Merrow, & Roenneberg, 2006). This implied, for instance, that late chronotypes would sleep out of phase relative to their circadian clock on workdays and in phase on work-free days. Assuming that DLMO is stable and represents the phase of the circadian clock, the phase angle difference between DLMO and sleep would be the only variable changing in this scenario. In support to this, a recent study and our own data have shown that the phase angle difference between DLMO and sleep onset was greater on work-free days relative to workdays (Paine & Gander, 2016). However, we also showed that DLMO is not stable in late chronotypes, suggesting that the circadian system of late chronotypes is remarkably flexible allowing them to shift their phase of entrainment between workdays and work-free days. This suggests that the negative health issues associated with social jetlag may not be a result of internal desynchronization (between the sleep phase and the clock phase), but rather a result of weekly shifts in phase of entrainment. Alternatively, it is possible that DLMO is not a reliable phase marker of the circadian clock. It is indeed likely that phase of entrainment of the circadian clock is stable (no shifts between workdays and work-free days), and that melatonin is an output of the clock that can relatively easily shift as the sleep-wake cycle does. In this case, the original concept of social jetlag would still hold.

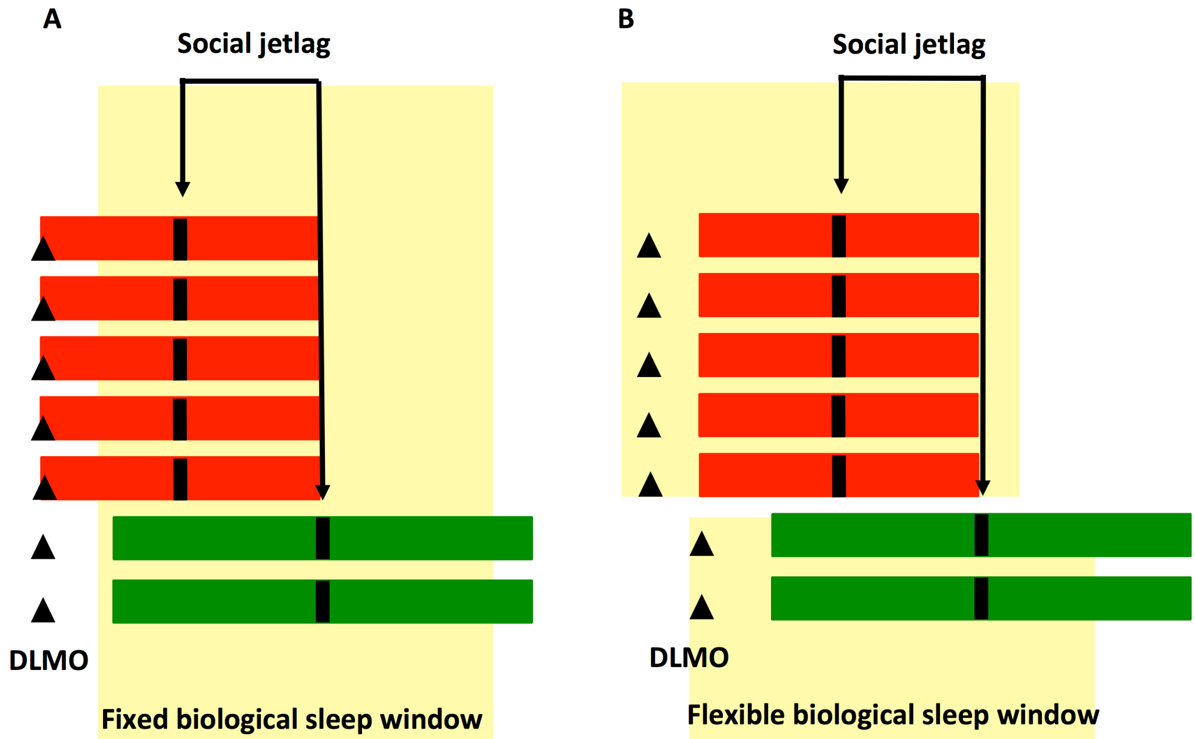

Figure 4. Two concepts of social jetlag.
Red bars represent sleep on workdays and green bars represent sleep on work-free days. The black vertical lines represent the midpoint of sleep on workdays (MSW) and on work-free days (MSF). Social jetlag is calculated as the absolute difference between MSW and MSF. Dim-light melatonin onset (DLMO) is represented by the black triangles. The yellow area shows the optimal biological sleep window that is determined by the circadian clock. In these conceptualizations DLMO is assumed to be a reliable indicator of the phase of entrainment of the circadian clock. A) Original concept of social jetlag: DLMO (phase of entrainment) is stable. During workdays someone suffering from social jetlag sleeps out of phase relative to his/her optimal biological sleep window. The negative health consequences associated with social jetlag are the result of not sleeping in phase with the circadian clock. B) Alternative concept of social jetlag: DLMO (phase of entrainment) is flexible, being earlier on workdays and later on work-free days. The negative health consequences associated with social jetlag are the result of weekly shifts in phase of entrainment.

Final remarks

In this thesis, I have shown how the circadian clock and its entrainment are challenged by modern society, leading to important handicaps in late chronotypes in terms of, for example, school performance. The influence of chronotype on school performance is complex, involving the interaction with many other factors. More studies are still needed to unravel the complex mechanisms explaining the poorer school performance in late chronotypes. We showed a time-of-day and subject-dependent effect of chronotype on grades. We hypothesized that chronotype mainly influences fluid intelligence (e.g. logic, reasoning, problem solving) since these are cognitive abilities required for scientific subjects where the effect of chronotype on grades was stronger. At which level of cognition and which changes occur in the brain when chronotypes are tested at a non-optimal time of day is an issue which needs further elucidation. In addition, it is not clear yet how cognitive abilities vary in different chronotypes considering the full 24-hours. The dichotomy that early chronotypes perform better in the morning and late chronotypes in the evening (synchrony effect) seems too simplistic for the complexity of the circadian system.

A better understanding of the relationship between chronotype and school performance would allow the scientific community to suggest effective changes in school policies. Simply delaying school starting times might not be the only or best solution. Activities at school to educate students about sleep and about the effects of light on the circadian clock should be implemented as well.

The idea that individuals are forced to perform at a non-optimal time of day should be also translated to the working population. There are many studies concerning students because it is easy to assess their performance by collecting grades. However, studies investigating, for instance, productivity of different chronotypes working at the same or different times of day would be of extreme interest for society.

Regarding the interventions to synchronize behavior to societal demands, we have focused on light. However, entrainment is a complex phenomenon and the circadian clock uses probably different internal and external time signals to maintain a stable phase of entrainment. Future studies should explore the role of other zeitgebers in human entrainment, also in combination with light. It is possible that some other time signals together with light exposure could enhance the phase shifting effects of light. For instance, physical activity alone (studies run in dim-light conditions) was able to facilitate re-entrainment following both advances and delays of the sleep-wake cycle (Barger, Wright, Hughes, & Czeisler, 2004; Miyazaki, Hashimoto, Masubuchi, Honma, & Honma, 2001). Another example is the consumption of caffeine in the evening that was recently shown to phase-delay circadian timing (Burke et al., 2015). It would be interesting to assess, for example, whether drinking coffee while being exposed to morning light could increase the advancing effects of light.

Even if the best combination of interventions were found, the problem of the great variability in individual responses to treatments remains. It is indeed very difficult to give general indications for interventions to adjust phase of entrainment. Light therapies should be tailored to the individuals based on their characteristics such as chronotype and sensitivity to light. There is a need to develop practical, quick, and precise circadian assessment tools. Melatonin is currently considered the best physiological phase marker to estimate phase of entrainment. However, we have shown that DLMO is surprisingly responsive in late chronotypes, raising the question whether DLMO may reflect plasticity in the clock outputs in response to transient changes in zeitgeber exposure. By knowing the exact circadian phase of an individual and his/her habitual light exposure profile, optimized light interventions in terms of timing, intensity and duration of the light pulse could be prescribed. Similarly, peak performance could be estimated and school/working schedules could become more flexible to accommodate individual needs.

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Summary

The rotation of the earth on its axis and around the sun determines regular changes in the environment, namely the alternation of day and night and of seasons. Nearly all organisms on earth have developed an internal time keeping mechanism (circadian clock) to synchronize (entrain) to the external light-dark cycle. Exposure to light is important in determining the phase of entrainment, but other individual factors such as sex, age, and genetic background play a role as well. As a result, humans can entrain with very different phases to the external light-dark cycle, giving rise to a wide distribution of chronotypes that ranges from early (larks) to late (owls) types.

Chronotype can be easily assessed with questionnaires (e.g Munich ChronoType Questionnaire; MCTQ) as the midpoint of sleep on work-free days (MSF), corrected for sleep debt accumulated on workdays (MSF_{sc}). Modern society often ignores this rich variety in sleep timing by imposing uniform (usually early) school and working starting times. As a result, the circadian clock of especially late chronotypes is often in conflict with the social clock, giving rise to a phenomenon called social jetlag (absolute difference between the midpoint of sleep on work-free days and on workdays).

The main objectives of this thesis were to describe the consequences that arise from the conflict between biological (circadian) and social clocks (part 1; chapters 2-5), to explore possible solutions to reduce social jetlag (part 2; chapter 6), and to better understand entrainment in real life conditions (part 3; chapters 7 and 8).

In **chapter 2** and **3** we investigated the role of chronotype and school attendance in relation to school performance in high-school students. Previous literature had shown that late chronotypes on average obtain worse school grades than early chronotypes. Here we showed that the chronotype-effect on grades depends on time of day (the effect is stronger in the morning) and on school subject (the effect is stronger for scientific subjects). In addition, we found that late chronotypes were more likely to be absent from class, and that absenteeism, in turn, was negatively associated with school performance. These findings suggest new hypotheses about how chronotype impacts school performance. Since late chronotypes sleep shorter on school days, insufficient sleep has always been considered a possible factor associated with lower school performance. However, our analysis showed that chronotype was a stronger predictor of school grades than sleep duration. The dependency on time of day of the chronotype-effect suggests that students with a late chronotype achieve lower grades particularly when tested at a non-optimal time of day (often late chronotypes are tested during their biological night). The dependency of the chronotype-effect on school subjects suggests that chronotype might influence specific cognitive abilities (e.g. fluid intelligence) that are important for scientific subjects.

In **chapter 4** we aimed to expand our previous results about the interaction effect between chronotype and time of day on grades. We assessed chronotype and collected grades in university students because they are examined early in the morning as well as late in the evening. Unfortunately, the distribution of grades over the course of the day was not uniform, with too few grades collected in the evening, which limited the interpretation of our results. Interestingly, also in this study, chronotype was associated with attendance (late chronotypes attended fewer lectures). Attendance and study effort were stronger predictors of grades than chronotype, suggesting that the chronotype-effect on grades is evident particularly in contexts were students are expected to attend classes early in the morning (most universities have more flexible schedules than schools).

In **chapter 5** we reviewed the literature about the relationship between chronotype and school performance, suggesting possible mechanisms behind a lower school performance in late chronotypes. Chronotype is likely to have both a direct and an indirect effect, with the latter effect being mediated by other factors important for school performance such as conscientiousness and motivation. The chapter ends with some suggestions regarding possible changes in school policies (e.g. tests scheduled later in the day) that would allow for testing all students on an even ground without any discrimination against late chronotypes.

In **chapter 6,** two light protocols were assessed for how well they could decrease social jetlag. The first study involved a decrease in evening light exposure by wearing blue-light-blocking glasses, and the second study involved an increase in morning light exposure by sleeping with open curtains. We found that filtering out blue light during the hours before going to sleep was associated with an advance in both sleep timing (on workdays) and in dim-light melatonin onset (DLMO). Sleeping with open curtains did not yield the same expected results at the group level, but the change in DLMO during the intervention week was associated with the increase of light in the bedrooms (participants who experienced a greater increase of light in their bedrooms showed a greater advanced in their DLMO). In both studies the effects were stronger during the first intervention week. Further studies are needed to determine the long-term effects of such interventions.

In both studies, we were not able to decrease the social jetlag of our participants. However, social jetlag at baseline was quite low (on average 1.5 hours), possibly leaving not enough room for improvement. Future studies should test the effectiveness of these or similar light interventions to decrease social jetlag in extremely late chronotypes who suffer from more than 2 hours of social jetlag.

In **chapters 7** and **8**, we aimed to better understand entrainment in real life conditions by assessing the influence of season (photoperiod) and weekly structure (work/school days vs. work-free days) on behavior (school attendance and performance), sleep, activity, and phase of entrainment (DLMO). We found that school attendance varied according to season with a peak in absenteeism in winter. Photoperiod was the strongest predictor of this seasonal variation in school attendance. Sleep, activity, and DLMO were mainly influenced by the weekly structure (not by season). All parameters were later on work-free days. While this is known for sleep (social jetlag), there is less evidence that DLMO also varies between workdays and work-free days. We found a delay in DLMO over the weekend and this was more pronounced in later chronotypes.

In conclusion, I have shown in this thesis how the circadian clock and its entrainment are challenged by modern society, leading to important handicaps in late chronotypes in terms of, for example, school performance. With our studies, we have increased our understanding of how chronotype impacts school performance and attendance by showing that the chronotype-effect on grades depends on time of day and school subject, and by showing that late chronotypes are more likely to be absent from class, which, in turn, lowers their school performance.

In this thesis, I have also reported the results of two studies that aimed to decrease the mismatch between the circadian and social clocks (social jetlag). Although social jetlag was not reduced, these findings show that simple 'in-home' light interventions are potentially effective in modifying phase of entrainment and sleep timing, confirming the results of previous laboratory studies about the effects of light on the circadian clock.

Finally I have shown how both season and weekly structure can influence entrainment in terms of behavior (school attendance and performance), sleep, activity and DLMO.

The findings of this thesis have important applications for society. Suggestions to improve school policies and practical solutions to delayed sleep have been developed. Most importantly, this work has generated several interesting hypotheses to be tested in future studies.

Samenvatting

Dutch translation by Renske Lok

Regelmatige wisseling tussen dag en nacht worden veroorzaakt door het draaien van de aarde rondom z'n as en de verandering van seizoen door het draaien van de aarde om de zon. Bijna alle organismen die op aarde leven hebben mechanismen ontwikkeld om de interne (circadiane) klok te synchroniseren (entraineren) met de externe licht-donker cyclus. Blootstelling aan licht is belangrijk om de fase van de klok vast te stellen, maar andere individuele factoren zoals geslacht, leeftijd en genetische achtergrond spelen ook een rol. Hierdoor kunnen mensen fasen hebben die erg verschillen van de externe dag-nacht cyclus. Dit zorgt voor een brede verdeling van chronotypes, variërend van vroege types (leeuwerik) tot laat (uil).

Chronotype kan het makkelijkst vastgesteld worden door middel van vragenlijsten (zoals de Munich Chronotype Questionnaire, MCTQ) die het middelpunt van slaap op werk-vrije dagen (MSF) bepaald. Dit wordt gecorrigeerd voor de slaapschuld die gedurende werkdagen wordt opgebouwd (MSF_{sc}). De hedendaagse maatschappij negeert vaak de rijke variatie in slaaptijden, door het opleggen van (vaak vroege) school- en werk starttijden. Het gevolg is dat de circadiane klok van voornamelijk late chronotypes vaak in conflict is met de sociale klok, wat resulteert in een fenomeen wat sociale jetlag wordt genoemd. Dit is gedefinieerd als het absolute verschil tussen middelpunt van slaap op werk-vrije dagen en op werkdagen.

Het doel van deze dissertatie was het beschrijven van de consequenties die ontstaan uit conflicten tussen de biologische (circadiane) klok en sociale klokken (deel 1; hoofdstuk 2-5), om een mogelijke oplossing te vinden om sociale jetlag te verminderen (deel 2; hoofdstuk 6) en een beter begrip te verkrijgen van het entraineren onder natuurlijke omstandigheden (deel 3; hoofdstuk 7 en 8).

In **hoofdstuk 2** en **3** hebben we onderzocht wat het verband is tussen chronotype en aanwezigheid op school in relatie tot schoolprestaties in middelbare scholieren. Literatuur heeft laten zien dat late chronotypes gemiddeld gezien slechtere cijfers halen in vergelijking met vroege chronotypes. Wij laten zien dat chronotype-effecten op cijfers afhangen van het tijdstip van de dag (het effect is sterker in de ochtend) en afhangen van het vak (het effect is sterker in wetenschappelijke vakken). Daarnaast hebben we gevonden dat late chronotypes minder aanwezig waren op school, en dat dit negatief geassocieerd was met schoolprestatie. Deze bevindingen leiden tot nieuwe hypothesen over hoe chronotype prestatie op school beïnvloed. Late chronotypen slapen bijvoorbeeld korter op schooldagen en onvoldoende slaap wordt aangeduid als mogelijke factor van slechtere prestaties. Echter, onze analyses lieten zien dat chronotype een sterkere voorspeller was van cijfers dan slaapduur. Het feit dat het tijdstip van de dag afhangt van het effect van chronotype op prestatie, suggereert dat studenten met een laat chronotype voornamelijk lagere cijfers halen wanneer ze moeten presteren op een niet-optimaal tijdstip van de dag (vaak worden late chronotypen getest gedurende hun biologische nacht). Het feit dat het chronotype-effect afhangt van het vak suggereert dat chronotype mogelijk effecten heeft op specifieke cognitieve aspecten (zoals 'fluid intelligence') die belangrijk is voor wetenschappelijke onderwerpen.

Het doel van **hoofdstuk 4** was om meer gegevens te verkrijgen over het interactie effect tussen chronotype en tijdstip van de dag op schoolcijfers. Daarvoor hebben we van universitaire studenten het chronotype vastgesteld en daarnaast cijfers van deze individuen verzameld. Universitaire studenten worden vaak in de ochtend of late avond getest. Helaas was de distributie van cijfers over de dag niet uniform, met weinig cijfers in de avond, wat de interpretatie van deze data limiteert. Opvallend is, dat ook in dit experiment, chronotype geassocieerd was met aanwezigheid (late chronotypes woonden minder colleges bij). Aanwezigheid en studietijd waren sterkere voorspellers van cijfers dan chronotype, wat suggereert dat het chronotype-effect op cijfers voornamelijk een rol speelt wanner college in de ochtend wordt gegeven (de meeste universiteiten hebben flexibelere schema's dan middelbare scholen).

Hoofdstuk 5 biedt een overzicht van de literatuur aan, waarin gekeken wordt naar de relatie tussen chronotype en schoolprestatie. Hierin worden mogelijke mechanismen gesuggereerd die het effect van chronotype op prestatie kunnen verklaren. Chronotype zal waarschijnlijk zowel directe als indirecte effecten hebben, waarin het laatste wordt gemedieerd door andere factoren die belangrijk zijn voor schoolprestaties, zoals bewustzijn en motivatie. Het hoofdstuk eindigt met suggesties om bijvoorbeeld het schoolbeleid aan te passen (bijvoorbeeld latere tijdstippen voor toetsen), wat ervoor zou zorgen dat alle studenten onder gelijkwaardige omstandigheden worden getest, zonder discriminatie jegens late chronotypes.

In **hoofdstuk 6** werden twee protocollen beschreven die beide licht gebruiken om vast te stellen of deze sociale jetlag konden verminderen. Het eerste experiment testte het effect van een afname in blootstelling aan licht in de avond door het dragen van een blauw-licht blokkerende bril. Het tweede experiment testte wat het effect was van een toename van de hoeveelheid licht in de ochtend door het slapen met open gordijnen. We vonden dat het filteren van blauw licht een paar uur voor bedtijd geassocieerd was met vroeger gaan slapen (op werkdagen) en dim licht melatonine onset (DLMO). Slapen met open gordijnen gaf niet het verwachte resultaat op groepsniveau, maar veranderingen in DLMO tijdens de interventie week waren geassocieerd met toename van licht in de slaapkamers (proefpersonen die een grotere hoeveelheid licht in hun slaapkamer hadden lieten een grotere vervroeging van hun chronotype zien). In beide studies waren effecten sterker gedurende de eerste interventie week. Meer experimenten zijn nodig om vast te stellen wat de lange termijn effecten van zulke interventies zijn.

In beide experimenten konden we sociale jetlag niet verminderen. Echter, sociale jetlag zonder interventie was relatief laag (gemiddeld 1.5 uur), mogelijk laat dit niet voldoende ruimte voor verbetering over. Toekomstige studies zouden de effectiviteit van deze of vergelijkbare licht interventies om sociale jetlag te verminderen in extreem late chronotype moeten testen, dit zijn proefpersonen die meer dan 2 uur sociale jetlag ervaren.

Het doel van **hoofdstuk 7** en **8** was om het entraineren in natuurlijke omstandigheden beter te begrijpen door vast te stellen wat het effect van seizoen (fotoperiode) en week structuur (werk/school dagen versus werk-vrije dagen) op gedrag (aanwezigheid op school en prestatie), slaap, activiteit en fase van entraineren (DLMO) zou zijn. Resultaten lieten zien dat aanwezigheid op school varieerde met seizoen, waarin er een piek in absentie was in de winter. Fotoperiode was de sterkste voorspeller van deze seizoensgebonden variaties in aanwezigheid op school. Slaap, activiteit en DLMO werden voornamelijk beïnvloed door de structuur van de week en niet seizoen. Alle gemeten parameters waren later op werk-vrije dagen. Dit was al bekend voor slaap (sociale jetlag), maar er was minder evidentie dat DLMO ook varieerde tussen werk- en werk-vrije dagen. We vonden een verlating van DLMO in het weekend en dit was meer uitgesproken in latere chronotypes.

Concluderend; ik heb in deze dissertatie laten zien hoe de circadiane klok en het entraineren uitgedaagd worden door de hedendaagse maatschappij, wat leidt tot belangrijke nadelen in latere chronotypes in dingen zoals schoolprestatie. Met onze experimenten hebben we meer begrip gekregen voor hoe late chronotype schoolprestaties en aanwezigheid beïnvloeden, waaruit blijkt dat effecten van late chronotype afhangen van het tijdstip van de dag en het vak, en dat late chronotypes vaker absent zijn wat weer leidt tot slechtere prestatie.

In deze dissertatie heb ik ook geprobeerd om de mismatch tussen het circadiane systeem en de sociale klok (sociale jetlag) te verminderen. Alhoewel sociale jetlag niet afgenomen was, laten mijn bevindingen zien dat simpele thuis interventies potentieel effectief kunnen zijn in het aanpassen van de fase van entraineren en timing van slaap. Dit bevestigd resultaten van eerdere laboratorium studies over effecten van licht op de circadiane klok.

Uiteindelijk heb ik laten zien dat zowel het seizoen als de week structuur het entraineren, in termen van gedrag (school aanwezigheid en prestatie), slaap, activiteit en DLMO, kunnen beïnvloeden.

De bevindingen van deze dissertatie hebben belangrijke toepassing in de maatschappij. Suggesties om schoolbeleid aan te passen en praktische oplossing om verlate slaap te vervroegen zijn ontwikkeld. Het belangrijkst is dat dit werk verscheidene interessante hypotheses heeft gegenereerd, die in toekomstige experimenten getest zouden moeten worden.

Sommario

La rotazione della terra attorno al suo asse e attorno al sole determina cambiamenti regolari nell'ambiente, ossia l'alternanza del giorno e della notte e delle stagioni. Quasi tutti gli organismi che abitano la terra hanno sviluppato un sistema interno (orologio biologico) capace di sincronizzarsi con il ciclo naturale del giorno e della notte. L'esposizione alla luce è importante nel determinare la fase di sincronizzazione, sebbene anche altri fattori, come il genere sessuale, l'età e il corredo genetico giochino un ruolo fondamentale. Di conseguenza, gli esseri umani sono in grado di sincronizzarsi con fasi molto diverse con il ritmo del giorno e della notte, dando così origine a una distribuzione di 'cronotipi' molto ampia che va dai tipi mattinieri (allodole) a quelli serali (gufi).

Il cronotipo è misurato attraverso l'utilizzo di semplici questionari (per esempio il Munich ChronoType Questionnaire; MCTQ) ed è definito come punto intermedio del periodo di sonno durante i giorni non lavorativi (weekend), corretto per il deficit di sonno che solitamente si accumula durante i giorni lavorativi. Spesso la società moderna non tiene conto di questa enorme varietà negli intervalli temporali in cui si dorme, dato che gli orari d'inizio di attività scolastiche e lavorative sono uguali (di solito presto la mattina) per tutti. Di conseguenza, l'orologio biologico (in particolare dei cronotipi serali) è spesso in conflitto con gli orari imposti dalla società, dando origine a un fenomeno chiamato 'jetlag sociale' (la differenza temporale tra il punto intermedio del sonno nei giorni lavorativi e non lavorativi).

Gli obiettivi principali di questa tesi consistono nel descrivere le conseguenze che possono derivare dal conflitto fra l'orologio biologico e quello sociale (parte 1; capitoli 2-5), esplorare possibili soluzioni per ridurre il jetlag sociale (parte 2; capitolo 6) e aumentare la comprensione del processo di sincronizzazione dell'orologio biologico con il ritmo del giorno e della notte nella vita di tutti i giorni (parte 3; capitoli 7 e 8).

Nei **capitoli 2** e **3** abbiamo indagato come il cronotipo e la presenza a scuola influenzano la prestazione scolastica in studenti di scuola superiore. Studi precedenti avevano mostrato che i cronotipi serali ottengono in media voti peggiori rispetto ai cronotipi mattinieri. Noi abbiamo rivelato che l'entità dell'effetto del cronotipo sui voti dipende dal momento della giornata (l'effetto è più marcato durante la mattina) e dalla materia scolastica (l'effetto è più marcato per le materie scientifiche). Inoltre, abbiamo scoperto che i cronotipi serali sono più spesso assenti dalla classe e che l'assenteismo è anch'esso associato negativamente con la prestazione scolastica. Questi risultati suggeriscono nuove ipotesi riguardo ai meccanismi attraverso cui il cronotipo influenza la prestazione scolastica. Dato che i cronotipi serali dormono meno durante la settimana, la mancanza di sonno è stata spesso considerata un fattore che poteva spiegare una prestazione scolastica peggiore. Tuttavia, i nostri risultati mostrano che il cronotipo è un fattore predittivo dei voti scolastici più influente della durata del sonno. Il fatto che l'effetto del cronotipo dipenda dal momento della giornata suggerisce che gli studenti con un cronotipo serale ottengono voti peggiori solamente quando le verifiche sono fatte in un momento non ottimale della giornata (spesso le verifiche sono programmate quando i cronotipi serali si trovano ancora nella loro notte biologica). Il fatto che l'effetto del cronotipo dipenda dalla materia scolastica suggerisce che il cronotipo possa influenzare abilità cognitive che sono particolarmente importanti per le materie scientifiche.

Nel **capitolo 4** ci eravamo posti l'obiettivo di sviluppare i risultati precedenti riguardanti l'interazione fra cronotipo e momento della giornata in relazione ai voti scolastici. Abbiamo misurato il cronotipo e raccolto i voti di studenti universitari, perché i loro esami sono svolti sia la mattina presto sia la sera tardi. Sfortunatamente, non siamo stati in grado di raccogliere un numero sufficiente di voti durante i diversi momenti della giornata (troppi pochi esami serali) e questo ha limitato l'interpretazione dei nostri risultati. Anche in questo studio, il cronotipo influenzava la presenza alle lezioni (i cronotipi serali avevano la tendenza a frequentare meno lezioni). Sia la presenza in classe che la motivazione a studiare sono risultati essere fattori predittivi dei voti più influenti del cronotipo, suggerendo che l'effetto del cronotipo sui voti è particolarmente evidente in contesti in cui agli studenti sia richiesto di frequentare le lezioni la mattina presto (molte università hanno orari più flessibili delle scuole).

Nel **capitolo 5** abbiamo svolto un'analisi critica della letteratura riguardante la relazione fra cronotipo e prestazione scolastica, con l'obiettivo di suggerire meccanismi che possano spiegare la prestazione scolastica peggiore tipica dei cronotipi serali. Il cronotipo sembra avere sia un effetto diretto sia uno indiretto sulla prestazione scolastica. L'effetto indiretto è mediato da altri importanti fattori come la coscienziosità e la motivazione dello studente. Il capitolo termina con diversi suggerimenti su come migliorare l'organizzazione del sistema scolastico (per esempio le verifiche potrebbero essere svolte più tardi) per permettere di valutare gli studenti in modo paritario senza discriminare quelli con un cronotipo serale.

Nel **capitolo 6** è stata valutata l'efficacia di due protocolli per ridurre il jetlag sociale. Nel primo studio, l'esposizione alla luce serale è stata diminuita tramite l'uso di speciali occhiali che bloccano il passaggio della luce blu. Nel secondo studio, l'esposizione alle prime luci della mattina è stata aumentata chiedendo ai partecipanti di dormire con le tende della stanza da letto aperte. I nostri risultati mostrano come la semplice riduzione dell'esposizione serale alla luce blu sia in grado di anticipare sia il momento in cui i partecipanti vanno a dormire (solo durante i giorni lavorativi) sia il momento in cui le concentrazioni di melatonina (ormone rilasciato durante il sonno) iniziano ad aumentare. Dormire con le tende aperte non ha condotto a risultati simili (al contrario di quanto ipotizzato). Tuttavia, i partecipanti che hanno sperimentato un aumento di luminosità maggiore nelle loro stanze sono gli stessi il cui ritmo della melatonina ha avuto un anticipo maggiore. In entrambi gli studi, gli effetti degli interventi erano più marcati durante la prima settimana, ponendo l'accento sul bisogno di nuovi studi per determinare l'efficacia a lungo termine di questi tipi di interventi.

In entrambi gli studi, non siamo stati in grado di ridurre il jetlag sociale dei nostri partecipanti. Tuttavia, il jetlag sociale di partenza era abbastanza basso (in media 1.5 ore), non lasciando troppe possibilità di miglioramento. Studi futuri dovrebbero testare l'efficacia nell'uso di terapie simili, basate sull'aumento/diminuzione dell'esposizione a fonti luminose, per ridurre il jetlag sociale in cronotipi serali che abbiano un jetlag sociale di almeno due ore.

Nei **capitoli 7** e **8** l'obiettivo principale era comprendere meglio come funziona il processo di sincronizzazione dell'orologio biologico con il ritmo del giorno e della notte nella vita di tutti i giorni. A tal scopo abbiamo analizzato l'influenza delle stagioni e della struttura della settimana (giorni lavorativi vs. giorni non lavorativi) su comportamento (prestazione e presenza scolastica), sonno, attività fisica e melatonina. Abbiamo scoperto che la presenza a scuola variava secondo la stagione con un picco nelle assenze registrato in inverno. La lunghezza della giornata era il fattore predittivo più influente di questa variazione stagionale. Sonno, attività fisica e melatonina erano per lo più condizionate dalla struttura della settimana (non dalle stagioni). Tutti i parametri misurati occorrevano più tardi durante i giorni non lavorativi. Mentre questo è risaputo per quanto riguarda il sonno (jetlag sociale), è meno chiaro che anche il ritmo della melatonina possa variare secondo il giorno della settimana (lavorativo o non lavorativo). Nel nostro studio, abbiamo mostrato per la prima volta che il ritmo della melatonina occorre più tardi durante il weekend (giorni non lavorativi) e che questo fenomeno è più marcato nei cronotipi serali.

In conclusione, gli studi raccolti in questa tesi dimostrano come l'orologio biologico e la sua sincronizzazione con il ciclo del giorno e della notte siano messi a dura prova dalla società moderna, portando i cronotipi serali a soffrire di importanti handicaps come per esempio nel caso della loro prestazione scolastica. I nostri studi hanno apportato un contributo fondamentale nella letteratura sulla relazione fra cronotipo e prestazione scolastica, mostrando che l'effetto del cronotipo dipende dal momento della giornata e dalla materia scolastica e mostrando che i cronotipi serali sono a maggior rischio di assenze scolastiche, fatto che a sua volta influisce negativamente sui voti.

In questa tesi, ho riportato anche i risultati di due studi che avevano l'obiettivo di ridurre il divario fra l'orologio biologico e quello sociale (jetlag sociale). Nonostante il jetlag sociale non sia stato diminuito, i nostri risultati mostrano come semplici accorgimenti riguardanti l'esposizione alla luce in casa possano modificare la fase di sincronizzazione e l'orario del sonno, confermando risultati precedenti di studi condotti in laboratorio riguardo agli effetti della luce sull'orologio biologico.

Infine, ho mostrato come sia le stagioni sia la struttura della settimana possano influenzare la sincronizzazione dell'orologio biologico in termini di comportamento (prestazione e presenza scolastica), sonno, attività fisica e melatonina.

I risultati di questa tesi hanno importanti applicazioni e ripercussioni per la società. Il nostro lavoro ha generato idee per migliorare il sistema scolastico e soluzioni per anticipare l'orario del sonno. Inoltre, i nostri risultati hanno sviluppato nuove interessanti ipotesi da verificare in studi futuri.

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Curriculum Vitae

Giulia Zerbini was born on the 8th of June 1987 in Trento, Italy. Her background is in Psychology. She obtained the Bachelor and Master degrees in Cognitive Psychology respectively in 2009 and 2011 at the University of Padova. From the first years of undergraduate studies it became clear to her that her passion was directed more to science rather than to the clinical work. Starting a PhD was therefore a straightforward decision. She wanted to complete her PhD abroad to develop herself both from a personal and professional point of view. In 2013 she joined the chronobiology research group in Groningen and became immediately passionate about this discipline. Studying circadian rhythms in humans allowed her to find immediate applications of her work in society. A great example is the collaboration with the high schools in Coevorden and Hardenberg that lead to her first publications and, as a consequence of her findings, to a delay in school starting times. During these years she learned several techniques to study sleep in the field, from actigraphy to assessing dim-light melatonin onset. She helped with teaching and supervised several high school, BSc and MSc students. Finally she participated to several national and international conferences to present her results. She would like to continue her research on sleep and circadian rhythms but with a focus on athletic performance.

Publications

Peer-reviewed

van der Vinne V*, **Zerbini G***, Siersema A, Pieper A, Merrow M, Hut RA, Roenneberg T, and Kantermann T. (2015). Timing of examinations affects school performance differently in early and late chronotypes. *Journal of Biological Rhythms*, 30(1): 53-60.
*These authors contributed equally to this work.

Zerbini G, van der Vinne V, Otto LKM, Kantermann T, Krijnen WP, Roenneberg T, and Merrow M. (2017). Lower school performance in late chronotypes: underlying factors and mechanisms. *Scientific Reports*, 7: 4385.

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In preparation

Zerbini G, Kantermann T, Roenneberg T, and Merrow M. Light interventions to decrease social jetlag.

Zerbini G, van der Vinne V, Otto LKM, Roenneberg T, Kantermann T, and Merrow M. Annual rhythms in school attendance and school performance.

Zerbini G, Roenneberg T, and Merrow M. Dim-light melatonin onset: the influence of season and weekly structure.