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; Prim, 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; Jelinková-Vondraková, 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.