

As Time Goes By: Effects of Basal Chronotype and School Timing on Chronotype Development During Adolescence

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Abstract

The misalignment between late chronotypes and early school start times affect health, performance and psychological well-being of adolescents. Here we test whether, and how, the basal chronotype (i.e. chronotype at the beginning of secondary school) and the school timing affect the magnitude and the direction of the developmental change in chronotype during adolescence. We evaluated a sample of Argentinian students (n=259) who were randomly assigned to attend school in the morning (07:45am-12:05pm), afternoon (12:40pm-05:00pm) or evening (05:20pm-09:40pm) school timings. Importantly, chronotype and sleep habits were assessed longitudinally in the same group of students along secondary school (at 13-14 y.o. and 17-18 y.o.). Our results show that: (1) although chronotypes partially align with class time, this effect is insufficient to fully account for the differences observed in sleep-related variables between school timings; (2) both school timing and basal chronotype independently affect the direction and the magnitude of chronotype change, with greater delays associated with earlier basal chronotypes and later school timings. The practical implications of these results are challenging and should be considered in the design of future educational timing policies to improve adolescents' well-being.

Introduction

Humans show close-to-24h (or circadian) rhythms in their behavior and physiology. These fluctuations are endogenous and coordinated by a main circadian clock, located in the suprachiasmatic nuclei¹. Even though humans are active during daytime, the expression of the endogenous circadian timing under specific external conditions (i.e. chronotype) shows variability among individuals^{2,3}. Chronotype has genetic basis⁴⁻⁶, but it also depends on other factors, such as age⁷⁻¹², light exposure^{2,13,14}, and cultural and social cues¹⁵⁻¹⁷. Consistently, individuals' chronotype ranges on a continuum between early and late types and can be assessed by evaluating behavioral¹⁸⁻²⁰ or physiological rhythms²¹⁻²³, as well as by using easy, reliable and standardized questionnaires. The widely used Munich Chronotype Questionnaire (MCTQ)²⁴ assesses several sleep-related variables including a chronotype proxy based on sleep timing: the sleep-corrected midpoint of sleep on free days (MSFsc). This marker highly correlates with other measures, such as the Morning-Eveningness Questionnaire²⁵⁻²⁷, the phase of actigraphy-evaluated sleep-rest activity rhythm^{18,19,24}, core temperature and other endogenous physiological rhythms^{21,22,28,29}.

Sleep timing approaches the individuals' endogenous tendencies only on free days¹⁸, as opposed to weekdays, where sleep timing is usually influenced by working and education schedules³. Accordingly, modern societies are characterized by the prevalence of a misalignment between the biological timing and the social timing (i.e. determined by social cues). This chronic condition is known as social jetlag (SJL)^{3,30}, which is calculated as the difference between the midpoint of sleep on free days and on weekdays^{3,31}. It is worth noting that SJL refers to differences on sleep timing but not on sleep duration (SD). Importantly, both SJL and short SD have been associated with negative consequences for physical and mental health, such as depression, higher rates of suicide, higher risk of substance abuse, obesity³¹⁻³⁶, and impaired cognitive performance^{37,38}.

Although adolescents' chronotype is particularly late⁷⁻¹², secondary school starts very early in the morning all around the world³⁹. This contrast between biological timing and social obligations is proposed to be the main cause of short SD on weekdays (SDw) and high SJL during adolescence^{33,34}. Consistently, delaying the school start time improves mood, wellbeing and academic performance⁴⁰⁻⁴⁴, and decreases diurnal somnolence and even the rate of vehicular accidents^{40,41, 45-47}. Altogether, these results suggest that a better alignment between adolescents' internal clock and school schedule might improve both their health and performance.

As a social cue, school start time might potentially modulate adolescents' chronotype. When comparing different morning school timings, results are not conclusive: a few studies found an association between later school start times and later chronotypes⁴⁸⁻⁵⁰, while others did not^{51,52}. However, when more distant school timings were compared (e.g. morning vs. afternoon), later school timings were associated with later chronotypes⁵³⁻⁵⁶. Unfortunately, the lack of random assignment of students to different school timings might confound and/or mask the effect of school timing on chronotype (due to biased assignment based on academic performance, socio-economic status or chronotype preferences). Although it has been well established that chronotypes become progressively delayed until the end of adolescence⁷⁻¹², whether and how this developmental effect is modulated by a social cue as school timing needs further investigation.

Recently, we partially addressed this point with a cross-sectional study comparing two well-defined age groups, younger and older adolescents randomly assigned to one of three different school timings at the beginning of secondary school: morning (07:45-12:05), afternoon (12:40-17:00) or evening (17:20-21:40)⁵⁷. Taking advantage of this unusual random assignment, we studied the relationship between school timing, chronotype and age⁵⁵. We found that, despite adolescents' chronotype was partially aligned with their school timing, students continued to experience high SJL and short SDw. Importantly, school timing modulates these effects: morning-attending students presented the earliest chronotypes, but the highest SJL and the shortest SDw⁵⁷. Our previous results also show an impact of age on chronotype, SJL and SDw, with larger differences between school timings for the oldest adolescents. The conclusions of this previous cross-sectional study regarding how the interaction between age and school timing affects chronotype are compelling, yet limited, because different students were evaluated at different time points during adolescence.

Here we present a follow-up study where the same group of students was evaluated at their 1st (13-14 y.o.) and their 5th (17-18 y.o.) year of secondary school. This longitudinal design allows us to assume that the differences observed between 1st and 5th year are due to age-related changes and not due to interindividual variability. First, we replicate, complement and strengthen the results obtained in our previous cross-sectional study. However the novelty and main aim of the present study is to understand which factors modulate the developmental change in chronotype that occurs during adolescence (Δ Chronotype, i.e. the variation in chronotype that occurs from 1st to 5th year of school) using a data-

driven approach. Importantly, the assessment of this aim is only possible with data corresponding to different time points for the same adolescents.

As mentioned before, it has been reported that chronotype is modulated by social cues¹⁵⁻¹⁷ and age^{7,8}, but some recent studies found low-to-moderate stability throughout adolescence in chronotype at individual level⁹⁻¹¹. This result means that even though chronotype delays on average, the developmental change (Δ Chronotype) is not necessarily the same for all students. The factors that explain this phenomenon are unknown. Here we propose that this change is modulated not only by school timing, but also by basal chronotype (i.e. the chronotype of each student in 1st year). The relationship between these two factors leads to four possible and alternative scenarios, where the magnitude and/or the direction of Δ Chronotype is modulated by school timing and/or each adolescent's basal chronotype (**Hypotheses box, Box Fig., Supp. Fig. 1**).

Although the four scenarios are possible, we know that: 1- Argentinian adolescents exhibit later chronotypes than adolescents from other countries^{7,8,53, 63-65}, and their basal chronotypes are particularly late; and 2- school timing, as a social cue, has been shown to modulate chronotype^{54,57}. Consistently, we think that the independent effects of both school timing and basal chronotype will better explain the age-related changes in chronotype (i.e. scenario 3). Importantly, as the interplay of late chronotypes and early school start times is expected to be the cause^{39,63} of unwanted sleep-associated conditions (e.g., short SD and high SJL^{39, 63-68}), we hypothesize that changes in social jetlag and sleep duration will be associated with Δ Chronotype and influenced by school timing. Particularly, we predict that a higher Δ Chronotype in morning-attending students will be associated with even higher SJL and lower SDw in 5th year, compared with other school timings. In summary, we expect that both school timing and basal chronotype will modulate the magnitude and direction of Δ Chronotype that, in turn, will be associated with changes in levels of SJL and SDw during adolescence.

Chronotype is progressively delayed during adolescence until it reaches a peak at the end of this developmental stage⁷⁻¹². Under comparable environmental cues (e.g. light-dark cycle), this chronotype delay has limits: either because of how much and at what age this 'delaying process' starts and ends during adolescence, or because the limits imposed by the intrinsic mechanism of the circadian clock^{17, 58-62}. Importantly, individuals will not be entrained to the environment outside these theoretical upper (and lower) limit of chronotype. The exact value of the upper limit is unknown and might depend on different environmental and social factors (e.g. light exposure, geographical longitude or latitude, culture, etc.). Beyond that, depending on the limit value, the magnitude and/or the direction of the developmental change in adolescents' chronotype will (or will not) be affected. For example, an extremely late upper limit will have no effect on adolescents' chronotype (because adolescents will not reach that upper limit, even at the end of secondary school).

The effect of school timing and/or basal chronotype, considering the previously mentioned upper limits, leads to four different scenarios which predict the magnitude and/or the direction of the developmental

changes of chronotype during adolescence.

1) The school timing affects the magnitude (not the direction) of Δ Chronotype independently of each student's basal chronotype (**Box Fig. a**). Earlier school timings will exert more pressure on the age-associated delay in chronotype. Thus, the magnitude of Δ Chronotype will be smaller in students attending the morning school timing than in students in the other school timings. Note that in this scenario the age-associated expected change does not exceed the upper limit of chronotype (**Supp. Fig. 1a**).

2) Only the basal chronotype, and not school timing, modulates the magnitude (but not the direction) of Δ Chronotype (**Box Fig. b**). Students with later basal chronotypes will reach the upper limit of the possible chronotypes range before their peers with earlier basal chronotypes. Consequently, the magnitude of Δ Chronotype will be smaller in students with later basal chronotypes, irrespective of school timings. Moreover, as students became older, chronotypes would be delayed for all school timings. Note that in this scenario the theoretical upper limit has to be lower than in scenario 1, affecting students' Δ Chronotype (**Supp. Fig. 1b**).

3) School timing and basal chronotype modulate the magnitude and direction of Δ Chronotype. Here, both phenomena act together but independently (**Box Fig. c**). On the one hand, later basal chronotypes will experience a smaller Δ Chronotype due to the reaching of the upper limit of possible chronotypes. On the other hand, school timing will also affect Δ Chronotype: earlier school timings will exert higher pressure and, consistently, students attending school earlier will experience smaller Δ Chronotype. Note that the school timing effect would take place considering the existence of the upper limit and, in the most extreme cases, it would lead to negative Δ Chronotype (i.e. morning attending students with late basal chronotypes will not delay, or will even advance, their chronotype, experiencing an earlier chronotype in their 5th year compared to their 1st) (**Supp. Fig. 1c**).

4) Both school timing and basal chronotype interact to modulate the magnitude and the direction of Δ Chronotype (**Box Fig. d**). In this scenario, the age-associated expected change in chronotype is within the range of possible chronotypes. Each school timing differentially affects Δ Chronotype depending on the basal chronotype. In particular, while the magnitude of the pressure exerted by morning and afternoon school timing is larger for later chronotypes, the evening school timing is late enough to not exert any pressure, regardless of basal chronotype (**Supp. Fig. 1d**).

Results

Mean chronotype and SDw depend on school timing and age, while SJL depends only on school timing.

To evaluate how school timing and age longitudinally affect chronotype during adolescence (data distribution in **Supp. Fig. 2**), we ran a linear mixed-effect model with chronotype (i.e. MSFsc) as the dependent variable, including school timing (morning, afternoon or evening), age (1st or 5th year) and their interaction as fixed effects, and students' id as a random effect (**Supp. Table 1, Supp. Table 2**). As in our previous cross-sectional results⁵⁷, we found a main effect of school timing ($F_{2,256}=29.697, P<0.0001$,

partial $\eta^2 = 0.188$, 90% confidence interval (CI)=0.119-0.256). Morning-attending students presented earlier chronotypes than both afternoon- and evening-attending students (Fig. 1a, **Supp. Table 3**), suggesting that school timing affects students' biological time, improving its alignment to the school timing where students were randomly assigned. We also found a significant main effect of age ($F_{1,256}=41.921$, $P<0.0001$, partial $\eta^2 = 0.141$, 90% CI=0.081-0.207), with earlier chronotypes in 1st year. Importantly, a significant interaction between school timing and age ($F_{2,256}=12.062$, $P<0.0001$, partial $\eta^2 = 0.086$, 90% CI=0.036-0.142) reveals that chronotype' changes throughout adolescence are modulated by school timing. At 1st year, adolescents' chronotype only slightly differed between school timings, but this difference gets larger by their 5th year (Fig. 1a). Consistently with our previous cross-sectional study, school timing modulates how adolescents' chronotype changes with age.

To evaluate whether the observed modulation was sufficient to fully, or only partially, align students' chronotype with their school schedules, we assessed the effects of age and school timing on both social jetlag (SJL) and sleep duration (SD) levels.

First, we ran a mixed effect model for SJL including school timing and age (data distribution in **Supp. Fig. 3**) as fixed factors and students' id as a random effect (**Supp. Table 4, Supp. Table 5**). School timing significantly affects SJL ($F_{2,256}=97.691$, $P<0.0001$, partial $\eta^2 = 0.433$, 90% CI=0.360-0.496), but we did not find significant effects neither for age ($F_{2,256}=0.194$, $P=0.660$, partial $\eta^2 = 0.001$, 90% CI=0.000-0.016) nor for the interaction between age and school timing ($F_{2,256}=2.288$, $P=0.104$, partial $\eta^2 = 0.018$, 90% CI=0.000-0.048)=. Particularly, morning-attending adolescents present significantly higher SJL levels (close to 3.5h) than their peers attending later school schedules (Fig. 1b, **Supp. Table 6**). In addition, afternoon-attending students present higher SJL levels than their evening-attending peers (2.18h vs. 1.74h), suggesting that afternoon school schedules also exert pressure on adolescents' sleep timing.

Second, we ran a mixed effect model for Sleep Duration (SD) including school timing, age and type of day (weekday or free day) (data distribution in **Supp. Fig. 4** and sleep timings in **Supp. Table 7**) as fixed effects and students' id as a random effect (**Supp. Table 8, Supp. Table 9**). We found significant main effects for school timing ($F_{2,256}=32.059$, $P<0.0001$, partial $\eta^2 = 0.200$, 90% CI=0.130-0.313), age ($F_{2,768}=35.032$, $P<0.0001$, partial $\eta^2 = 0.044$, 90% CI=0.023-0.070) and type of day ($F_{2,768}=392.264$, $P<0.0001$, partial $\eta^2 = 0.338$, 90% CI=0.276-0.379). This last effect indicates that students sleep less on weekdays, as expected. Additionally the interactions between type of day and age ($F_{2,768}=10.117$, $P=0.002$, partial $\eta^2 = 0.013$, 90% CI=0.003-0.029) or school timing ($F_{2,768}=90.161$, $P<0.0001$, partial $\eta^2 = 0.190$, 90% CI=0.150-0.230) were also significant. Conversely, the interaction between age and school timing ($F_{2,768}=1.998$, $P=0.136$, partial $\eta^2 = 0.005$, 90% CI=0.000-0.015) and the triple interaction between age, school timing and type of day ($F_{2,768}=1.368$, $P=0.255$, partial $\eta^2 = 0.004$, 90% CI=0.000-0.012) were not significant. On weekdays, students sleep less when they are older and morning-attending students sleep less than adolescents with later school schedules (Fig. 1c). Instead, students do not differ in their sleep duration, despite their age or school schedule, on free days (Fig. 1c, **Supp. Table 10**).

Thus, morning-attending students present very short SDw (i.e. high levels of sleep loss) in their 1st year of school and this situation aggravates as adolescence progresses. The difference in SDw between school timings was not compensated by napping (**Supp. Fig. 5, Supp. Table 11, Supp. Table 12, Supp. Table 13**): even considering naps, morning-attending students do not reach the recommended 8h of sleep^{69–71}. Altogether, the results presented here support that school timing modulates only partially adolescents' chronotype with both SJL and SDw levels depending on the school timing to which students were randomly assigned at the beginning of secondary school.

The developmental change on chronotype during adolescence depends on both school timing and basal chronotype. In the previous section, we showed that mean changes in chronotype depend on age and school timing. However, the association between 1st and 5th year's chronotypes is low-to-moderate in all school timings (morning: $t=3.462$, $p=0.001$ $r=0.344$, 95% CI=0.149-0.514; afternoon: $t=1.769$, $p=0.080$ $r=0.182$, 95% CI=-0.022-0.372; evening: $t=4.439$, $p<0.0001$ $r=0.461$, 95% CI=0.261-0.623) (**Supp. Fig. 6**). Here, we propose that basal chronotype (i.e. 1st year chronotype) might explain this lack of stability. Consistently, we contrasted the four scenarios previously described (**Hypothesis box, Supp. Fig. 1**) to evaluate whether basal chronotype and school timing affect the developmental changes in chronotype (i.e. age-related changes in chronotype or Δ Chronotype).

Basal chronotype tertiles suggest that the developmental change in chronotype depends on basal chronotype, with adolescents in the earliest tertile delaying their chronotypes the most during secondary school (Fig. 2a, **Supp. results 1**). We ran a linear regression model with Δ Chronotype (MSFsc 5th year - MSFsc 1st year) as the dependent variable, and both school timing and basal chronotype, as predictors (**Supp. Table 14, Supp. Table 15**). Δ Chronotype was affected by both school timing ($F_{2,253}=19.678$, $P<0.0001$, partial $\eta^2 = 0.135$, 90% CI=0.073-0.198) and basal chronotype ($F_{1,253}=160.343$, $P<0.0001$, partial $\eta^2 = 0.388$, 90% CI=0.314-0.455). Δ Chronotype was smaller for later than for earlier basal chronotypes, meaning that chronotype becomes less delayed with age for later basal chronotypes (Fig. 2b). For all school timings, the slopes of the relationship between the Δ Chronotype and the basal chronotype were different to zero (Morning: $b=-0.711$, 95% CI=-0.888 to -0.534, $t=-7.909$, $P<0.0001$; Afternoon: $b=-0.823$, 95% CI=-1.009 to -0.637, $t=-8.717$, $P<0.0001$; Evening: $b=-0.533$, 95% CI=-0.738 to -0.327, $t=-5.109$, $P<0.0001$). However, the interaction between basal chronotype and school timing was non-significant ($F_{2,253}=2.150$, $P=0.119$, partial $\eta^2 = 0.017$, 90% CI=0.000-0.037), showing that slopes did not differ between school timings. Thus, the magnitude of Δ Chronotype similarly depends on the basal chronotype for the three school timings. For example, a 1st year afternoon-attending student with a basal chronotype equal to the mean for the afternoon school timing (MSFsc=06:07) would delay their chronotype by 61min by the time s/he gets to 5th year. However, a same-class peer with a basal chronotype of 07:07 (1h later) would only delay it 12min (i.e. the difference between these students' Δ Chronotype is 49min, which is the slope of the model for the afternoon).

Our results are consistent with scenario 3: both school timing and basal chronotype additively affect Δ Chronotype during adolescence, with no interaction between them. Even though morning-attending

students experienced, on average, a lower delay in their chronotype from 1st to 5th year (compared with their afternoon- and evening-attending peers), overall, students with earlier basal chronotypes exhibited larger delays and those with later chronotypes showed smaller delays or advances, regardless of their school timing.

Age-related changes on SJL and SDw are associated with Δ Chronotype.

Later chronotypes are associated with higher levels of social jetlag (SJL) and a lower sleep duration on weekdays (SDw), especially when attending school in the morning^{39, 63–68}. Here we explored whether the individual changes in SJL or SDw during adolescence depend on Δ Chronotype and/or school timing. We ran a linear regression model with the age-related changes on SJL (i.e. Δ SJL=SJL 5th year - SJL 1st year) (**Supp. Fig. 7**) as the dependent variable and Δ Chronotype and school timing as predictors (**Supp. Table 16, Supp. Table 17**). We found significant main effects of both school timing ($F_{2,253}=4.493$, $P=0.012$, partial $\eta^2=0.034$, 90% CI=0.004-0.075) and Δ Chronotype ($F_{2,253}=235.795$, $P<0.0001$, partial $\eta^2=0.482$, 90% CI=0.413-0.543). In brief, the more delayed the chronotype becomes from 1st to 5th year, the bigger the change in SJL. For example, if a hypothetical afternoon-attending student exhibits a Δ Chronotype equal to the mean change for their school timing (Δ Chronotype=61min, e.g. from 05:00 to 06:01), their SJL will increase by just 2min. However, another student, with a 1h-larger Δ Chronotype (e.g. from 05:00 to 07:01, i.e. 121min), would increase their SJL on 35min along secondary school. Importantly, the interaction between Δ Chronotype and school timing was significant ($F_{2,253}=7.021$, $P=0.001$, partial $\eta^2 = 0.053$, 90% CI=0.014-0.100). The association between Δ SJL and Δ Chronotype was progressively weaker the later the school timing, even though the comparison between afternoon and evening school timings was not significant (**Fig. 3a, Supp. Table 18**). Morning-attending students exhibit larger changes in SJL for a given Δ Chronotype, compared with their afternoon- and evening- attending peers (slope comparisons: morning vs. afternoon: $t=2.767$, $P=0.017$; morning vs. evening: $t=3.552$, $P=0.001$).

Age-related changes in SDw also showed interindividual differences (**Supp. Fig. 8**), even though changes on mean SDw were similar when comparing school timings (**Fig. 1c**). We ran a linear regression model with the age-related changes in SDw (Δ SDw=SDw 5th year - SDw 1st year) as the dependent variable, and Δ Chronotype and school timing as predictors (**Supp. Table 19, Supp. Table 20**). As expected, the main effect of school timing was non-significant ($F_{2,253}=1.433$, $P=0.241$, partial $\eta^2 = 0.011$, 90% CI=0.000-0.037), indicating that SDw change similarly in different school timings (**Fig. 1c**). We found a significant main effect of Δ Chronotype ($F_{1,253}=8.196$, $P=0.0046$, partial $\eta^2 = 0.031$, 90% CI=0.006-0.075) and, importantly, a significant interaction between Δ Chronotype and school timing ($F_{2,253}=7.852$, $P<0.001$, partial $\eta^2 = 0.058$, 90% CI=0.017-0.108), indicating that school timing modulates the effect of Δ Chronotype on age-related changes in SDw. In particular, afternoon- and evening-attending students with larger delays in their chronotype throughout adolescence exhibit less shortening, or even a lengthening, of their SDw (afternoon: $b=0.191$, 95% CI=0.013-0.369, $t=2.108$, $P=0.036$; evening: $b=0.515$, 95% CI=0.278-0.756, $t=4.281$, $P<0.0001$) (**Fig. 3b**). To illustrate, an average afternoon-attending student (Δ Chronotype=61min) would decrease their SDw by 44min, while a peer with a 1h-larger Δ Chronotype (i.e.

121min) would decrease their SDw by 32min. Note that the corresponding slope is the difference between 44min and 32min, which is 12min. On the other hand, morning-attending students with the greatest delays in their chronotypes by their 5th year, showed a tendency to shorten their SDw the most, although the slope was not different from zero ($b=-0.109$, 95% CI=-0.310-0.093, $t=-1.062$, $P=0.289$). Despite the fact that both the slopes for afternoon- and evening-attending students did differ from zero, only evening and morning slopes significantly differ between them (morning vs. evening: $t=-3.950$, $P<0.001$) (**Supp. Table 21**). Even though one would expect that age-related chronotype delays in morning-attending adolescents would be strongly associated with a comparable increase in SJL and decrease in SDw^{3,54,72-74}, our results show that SJL increases accordingly with the chronotype delay while SDw did not decrease as much as expected.

Discussion

Here we achieved two related and complementary aims. First, we reproduced and strengthened our previous cross-sectional results⁵⁷ on how school timing and age affect chronotype and sleep. Second, we showed that the magnitude and the direction of the age-associated change on chronotype depends on both school timing and basal chronotype (i.e. chronotype of adolescents in their 1st year of secondary school).

Consistently with our previous data⁵⁷, we found that students' chronotypes were partially aligned with their school timing. Chronotype depends on both school timing and age, as well as on their interaction: the midpoint of sleep on free days (MSFsc) is later in older adolescents and later school timings, with larger differences between school timings for older adolescents. Social jetlag (SJL) is higher and sleep duration on weekdays (SDw) is shorter when school timing is earlier, especially for older students attending school in the morning. Most results were consistent between both studies, reinforcing our conclusion that school timing, as a social cue, partially modulates adolescents' internal timing.

Importantly, our longitudinal design allowed us to further analyze the low-to-moderate stability of chronotype during adolescence reported in previous longitudinal studies⁹⁻¹¹. We found that Δ Chronotype depends on basal chronotype. A previous study reported that chronotype development was modulated by the interaction between age and circadian preferences⁹ finding similar results. However, basal chronotype as a factor that contributes to the low stability of chronotype along development has not been previously reported and, thus, our approach and results contribute to understand how chronotype changes during adolescence.

Previous works have studied how chronotype is affected either by school timing^{7,8,61} or by age^{7-12,59} during adolescence, but here we analyzed these two factors together and longitudinally. Our results are consistent with our prediction that basal chronotype and school timing have independent and additive effects on adolescents' chronotype (Supplementary Fig. 1c, Scenario 3): 1- later school timings are associated with later chronotypes, with a stronger association in older adolescents, and 2- earlier basal chronotypes experience a bigger Δ Chronotype. According to our model, while a morning-attending

student with a basal chronotype equal to 05:46 (i.e. the mean basal chronotype for this school timing) practically does not change their chronotype during secondary school, 1-hour later basal chronotypes (i.e. 06:46) would advance their chronotypes 45min by 5th year. Thus, morning-attending adolescents who have late basal chronotypes at the beginning of secondary school might experience smaller delays or even advance their chronotype with age. As summarized in Scenario 3, getting older is not necessarily associated with later chronotypes.

Here we propose a mechanism that includes the existence of limits to Δ Chronotype during adolescence. Particularly, an upper limit associated with either the developmental stage achieved at the end of secondary school^{58,59} or with the entrainment mechanism of the circadian clock⁶¹ might explain the effects of both school timing and basal chronotype on Δ Chronotype. Previous works showed that chronotype variability among adolescents depends on different factors, including genetics, culture, light exposure, schedules and age^{2, 4-8, 12-17, 57}. On the one hand, advanced pubertal stages have been associated with later chronotypes^{58,78}: students with later basal chronotypes might be the ones presenting the most advanced pubertal stages at the beginning of secondary school. If this case, they would reach the upper limit before their peers and, consistently, they would have a smaller delay in chronotype between 1st and 5th year than they peers who started secondary school at lower pubertal stages. On the other hand, the upper limit might be associated with the entrainment mechanism. To be entrained to the external 24h light-dark cycle, humans have to be exposed to light at specific times of the day^{2, 17, 61}. Consistently, the interindividual variability exists but has limits and the range of chronotypes does not cover the 24h (i.e. some theoretically possible chronotypes might not be compatible with entrained rhythms⁶¹). Although humans can invert their wake-sleep cycle to be active at night and sleep during the day, as individuals who work night shifts, these subjects do not exhibit stable entrained rhythms^{60, 62, 79}. As chronotype is delayed throughout adolescence^{7, 8, 10, 11}, students with later basal chronotypes would reach the upper limit of stable entrainment⁸⁰ before their earlier peers. Furthermore, in our setting, this scenario is especially plausible because Argentinian adolescents exhibit particularly late basal chronotypes^{7, 8, 53, 57, 80, 81}. Based on our data, we cannot disambiguate whether the upper limit of chronotype exists and depends on the developmental stage and/or on the entrainment mechanisms of the circadian clocks. Thus, future work is needed to fully understand the causes of the association we found between basal chronotype and Δ Chronotype during adolescence.

Importantly, our results show not only that Δ Chronotype depends on basal chronotype and School timing but also that Δ Chronotype is associated with age-related changes in SJL and SDw. As expected, large delays in chronotype were associated with an increase in SJL and this association progressively weakens from morning to evening school timings (**Supp. Discussion**). In contrast, a delay in chronotype was associated with an increase in SDw in both afternoon and evening school timings, with a steeper association for the latter, and we found no association in the morning (**Supp. Discussion**). Thus, a better alignment between adolescents' internal timing and school timing seems to be beneficial in terms of sleep duration for afternoon- and evening-attending adolescents but not for their morning-attending peers. The latter was surprising because, although most adolescents shorten their SDw from 1st to 5th

year, the difference on SDw does not depend on their Δ Chronotype. A possible explanation for this result is that morning-attending students were already sleep-deprived in their 1st year and, consequently, their SDw might not be further shortened because of homeostatic reasons. Thus, the effect of a delay in Chronotype in morning-attending students is mostly absorbed by an increased in SJL levels and not by a shortening on SDw.

This study has several limitations. First, chronotype and sleep-related variables were self-reported through standardized questionnaires. Consistently, we cannot rule out a bias in students' answers, but they are highly improbable because students were blind to our experimental hypotheses. However, objective assessment of sleep and chronotype, such as actigraphy, could be more suitable. Second, our analyses are based on regressions, which do not allow us to establish causality but only association among variables. Third, we did not have access to other predictors that might modulate chronotype and its developmental change, such as pubertal stage, socioeconomic status, the usage of medications, the presence of illnesses, etc. Fourth, in this longitudinal study we only have data from two time points, one at the first and another at the last year of secondary school. The inclusion of additional time points (e.g. in the middle of secondary school) would be preferable but it was impossible due to operative reasons. Finally, the lack of assessment of chronotype and sleep habits before the beginning of secondary school does not allow us to unequivocally affirm that the initial point was completely balanced between school timings, even considering the random assignment.

This research also has some important strengths. First, the longitudinal design allows us to study the developmental changes during adolescence. Second, the sample size of our study is one of the highest among similar studies^{53,54,56,82,83}. Third, as in our previous study, we worked with three different school schedules, including an evening school timing (17:20 – 21:40). Finally, the random assignment of students to a particular school schedule at the beginning of their secondary school, allowed us to assume no differences in chronotype and sleep habits between school timings before starting secondary school.

Our results have several practical implications. First, we found that an early morning but also the afternoon school timing is associated with unhealthy sleep habits in adolescents. Consistently, a practical implication when thinking about better school start times would be that later morning school starting times might help but may not be enough for adolescents to have healthy sleep. This is especially relevant in populations exhibiting particularly late chronotypes, such as the adolescents from Argentina, Uruguay and Spain^{54,84}. In these situations, an evening school timing might be at least considered by the educational community and/or policy makers. Many families and even the educational community believed that the morning school timing is 'the most favorable school timing', but our work and several others support the idea that it is not the case: afternoon (or evening) school timing might be a more equitable and even preferable environment where early chronotypes do not present an advantage over late ones^{53,55,57}. Second, we show that school timing modulates chronotype and sleep habits during adolescence and, then, the undesirable conditions or behaviors associated with eveningness reported in the literature (e.g. depressive feelings or substance use) might be associated with the lack of alignment between chronotype and school timing (previous studies only include students that attend school in the

morning^{10,11,85-90}). Thus, future studies should include the effect of school timing. Third, the low-to-moderate stability observed in chronotype during adolescence suggests that chronotype is a malleable target for interventions^{10,11,85-90}: knowing that earlier basal chronotypes would exhibit larger delays, provides us new insights to help design interventions addressing adolescents sleep health and behavior.

Finally, the association between basal chronotype and the magnitude and direction of Δ Chronotype reported here go against the most parsimonious and intuitive notion that all adolescents would similarly delay their chronotype. Chronotype does not necessarily delay during adolescence and it is modulated by basal chronotype and school timing. These results might modify the previously suggested policies to improve the alignment between school timing and adolescents' internal timing. For example, the assignment of school timing based only on basal chronotype would not be as beneficial for adolescents' sleep health and academic performance^{53-55,57} as expected. Of course, more evidence is needed to shed light on this matter and to understand their practical implications, but this novel finding adds knowledge to the field and opens a new range of possibilities and questions. Exploring the underlying mechanisms of both school timing and basal chronotype effects on how chronotype changes during adolescence will lead us to a better understanding of how we can help adolescents to reach healthier sleep habits.

Methods

Ethical approval. The study and all the methods included on it were conducted in accordance with relevant guidelines and regulations, including the ethical recommendations for human chronobiological research⁹¹ and Argentinian national regulations. The study was not invasive of the integrity of the participants and the data was collected during regular school hours. The study was approved by the institutional Ethical Committee of the Universidad Nacional de Quilmes (Verdict #4/2017) and by the head of school. Written informed consent was obtained from the head of the school. Students provided oral informed consent to participate.

Participants. This study was performed in two different moments (June 2015 and July 2019) at a local secondary school in the City of Buenos Aires, Argentina (34° 60' S, 58° 38' W). The school year starts in March and ends in December in Buenos Aires thus, the data was collected after three/four months of classes. All of the students who attended school on the corresponding day of data collection and were at 1st year (i.e. 2015) or at 5th year (i.e. 2019), were invited to participate in the study. The attendance percentage was higher than 75% on each school timing and year (2015: morning, 97.50%; afternoon, 90.24%; evening, 87.01%. 2019: morning, 75.35%; afternoon, 79.11%; evening, 91.23%) and no student refused to participate. From the 436 and 352 students who completed the questionnaire in their 1st and 5th year, respectively, 259 students were included in the analyses performed in this study. Only those students who participate on the study on both years, who maintained their original school timing and with complete data on both years were included. The final sample of students was balanced on gender (50.97% females) and it was age-homogeneous (1st year: M=13.49 y.o., SD=0.33 ; 5th year: M=17.58 y.o., SD=0.33).

Procedure. A crucial aspect of our experimental setup is that in this particular school, the school timing (morning, 07:45–12:05; afternoon, 12:40–17:00; evening, 17:20–21:40) is set by a lottery system at the beginning of the secondary school, as described in depth in our previous study⁵⁷,

Briefly, in June 2015 students in their first school year filled a questionnaire including demographic information (date of birth and self-defined gender) and the Spanish version of the MCTQ²⁴. MCTQ includes questions about sleep habits and results in a local time point (MSFsc) where earlier times (i.e. low values) indicate early chronotypes and late times (i.e. high values) indicate late chronotypes²⁴. Data collection was performed during students' typical school hours (morning, afternoon and evening school timings). Data collection and analysis were not performed blind to the conditions of the experiments. The exact same procedure was applied in both June 2015 (during their first school year) and July 2019 (during their last school year).

Measurements. For each student on each school year, we obtained a chronotype index: the sleep-corrected midpoint of sleep time on free days (MSFsc)²⁴, social jetlag (SJL) and sleep duration on both week (SDw) and free days (SDf). From these measurements we also calculated the Δ Chronotype (i.e. developmental change in chronotype, $MSFsc_{5th\ year} - MSFsc_{1st\ year}$), the Δ SJL (i.e. $SJL_{5th\ year} - SJL_{1st\ year}$) and the Δ SDw (i.e. $SDw_{5th\ year} - SDw_{1st\ year}$).

Not all the variables were obtained for all students. Missing values occurred when a variable could not be calculated because of incomplete information (i.e. when a student did not complete all of the MCTQ questions). The data from a student was only included if the information was enough to calculate at least MSFsc, SJL, SDw and SDf. Missing data were omitted from the analyses.

Statistical analysis. All statistical analyses were performed using the R system for statistical computing (v.4.0.2; R Core Team, 2020).

We ran linear mixed-effect models to determine whether school timing (morning, afternoon or evening) and age (1st or 5th school year) were associated with MSFsc or SJL. For sleep duration, the linear-mixed model included type of day of the week (weekdays or free days), school timing (morning, afternoon or evening) and age (1st or 5th school year). The same analysis was performed for total sleep duration (nocturnal sleep + naps). Students ID was included as a random effect in every model. P-values were computed using lmerTest package⁹².

We ran a generalized linear models to test whether the developmental change in chronotype depends on school timing (morning, afternoon or evening) and basal chronotype (i.e. chronotype in 1st year) and to evaluate whether age-related changes in SJL and SDw depend on school timing (morning, afternoon or evening) and on the developmental change in chronotype.

Normality of the residuals of the models was checked using Kolmogorov–Smirnov tests. Student's t-tests were used to perform post-hoc pairwise comparisons for categorical variables. We used an alpha level of

0.05 for all of the statistical tests. When applicable, we used Bonferroni correction for multiple comparisons (corrected $P < 0.05$). Partial η^2 effect sizes were computed *using sjstats package* version 0.18.0.

Declarations

Data and code availability

The data and code that support the findings of this study are available from the corresponding author on request.

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Author contributions

Conceptualization: G.R.F, A.P.G., M.S. and M.J.L. Data collection: G.R.F, A.P.G. and M.J.L. Data analysis: G.R.F. and M.J.L. Interpretation: G.R.F, A.P.G., M.S. and M.J.L. Writing: G.R.F, A.P.G. and M.J.L., with revisions from all other authors.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Four theoretical scenarios of the effect of school timing and basal chronotype (in 1st year of high school) on Δ Chronotype (MSFsc 5th year – MSFsc 1st year). a- Only school timing has an effect. b- Only the basal chronotype has an effect. c- Basal chronotype and school timing have additive effects. d- Basal chronotype and school timing interact. The graphs on each scenario represent the expected developmental change in chronotype (i.e. Δ Chronotype) as a function of the basal chronotype (i.e. 1st year MSFsc) for each school timing. A zero value on the vertical axis indicates no change in chronotype from 1st to 5th year. Positive or negative values indicate that chronotype is delayed or advanced, respectively, in 5th compared to 1st year. Each colored line represents the linear relation between Δ Chronotype and basal Chronotype for each school timing. Grey arrows represent Δ Chronotype for three representative basal chronotypes (early, intermediate and late), the base of the arrows represent students' chronotype in their 1st year (i.e. the basal chronotype) and the arrowheads represent students' chronotype in their 5th year.

Figure 2

Longitudinal changes in Chronotype, Social jetlag and Sleep duration during adolescence. a - Mean changes on Chronotype depend on school timing and age. Evening-attending students exhibit later MSFsc than their morning-attending peers: 47min in 1st year (06:33 vs. 05:46) and it doubles to 104min

in 5th year (07:28 vs. 05:44). Afternoon-attending students show a similar pattern in 5th year: 82min later MSFsc than their morning-attending peers (07:08 vs. 05:46). Post-hoc pairwise comparisons, $p < .006$ (Bonferroni-corrected $p < .05$). b- SJL depends on school timing. SJL levels are lower for evening-attending students than for their afternoon-attending peers, both in 1st year (1.68h vs. 2.16h) and in 5th year (1.80 vs. 2.20). The same happens when compared to morning-attending students, who present the highest SJL levels (3.70h and 3.40h in 1st and 5th year, respectively). No significant differences were found between 1st and 5th year at any school timing. Post-hoc comparisons, $p < .017$ ($p < .05$, Bonferroni corrected). c- School timing and age affect sleep duration on weekdays (SDw) but not on free days (SDf). On weekdays, adolescents sleep less in their 5th year regardless of their school timing, and in the morning school timing regardless of their age. Students sleep more on free days than on weekdays independently of their age and school timing. No differences were found between school timings and age on free days. The interaction between school timing and age was not significant. The asterisk (*) indicates significant difference in sleep duration between 1st and 5th year across school timings, which was found on weekdays but not on free days. Post-hoc comparisons, $p < .0038$ ($p < .05$, Bonferroni corrected). Data are Mean \pm s.e.m. N=259. Lowercase letters indicate significant differences between groups: a, compared with morning of the same school year; b, compared with afternoon of the same school year; c, compared with evening of the same school year; d, compared with 1st year of the same school timing; e, compared with 5th year of the same school timing; f, compared with morning, across age groups; g, compared with afternoon, across age groups; h, compared with evening, across age groups.

Figure 3

Developmental changes in chronotype (Δ Chronotype) during adolescence depend on both school timings and students' basal chronotype. a- Basal chronotype tertile affects the change in Chronotype from 1st to 5th year. Those students on the earliest tertile delayed their chronotype by their 5th year, independently on school timing (from 04:11 to 05:08, from 04:47 to 06:38, from 05:08 to 06:58, for morning-, afternoon- and evening- attending students respectively). Students on the latest tertile of MSFsc, on the other hand, did not delay or even advanced their chronotypes (from 07:20 to 06:07, from 07:34 to 07:29, from 07:59 to 08:03, for morning-, afternoon- and evening- attending students respectively). b- Δ Chronotype depends on basal chronotype and school timing. Students with a late basal MSFsc experienced a lower chronotype change from 1st to 5th year. Particularly, the slope of the relationship between Δ Chronotype and basal chronotype indicates that, when the basal chronotype is 1-hour later, the age-related changes on chronotype are lower: 43min (95% CI=-53 to -32min), 49min (95% CI=-61 to -38min) and 32min (95% CI=-44 to -20min) for morning-, afternoon- and evening-attending students, respectively. N=259. Δ Chronotype=MSFsc 5th year - MSFsc 1st year. Basal chronotype=MSFsc in 1st year. Color indicates school timing: yellow, green and blue for morning, afternoon and evening, respectively.

Figure 4

Age-related changes in social jetlag and Sleep duration on weekdays are associated with developmental changes in chronotype. a - Δ SJL (SJL 5th year - SJL 1st year) highly correlates with Δ Chronotype (MSFsc 5th year - MSFsc 1st year). Higher increases in SJL levels are associated with larger delays on chronotype from 1st to 5th year (i.e. slopes): morning-, afternoon- and evening-attending students experience an increase of 48min (95% CI=40-56min), 33min (95% CI=26-40min) and 26min (95% CI=16-35min), respectively, in SJL for each hour that their chronotype is delayed. b - Δ SDw (SDw 5th year - SDw 1st year) association with Δ Chronotype depend on school timing. For morning-attending students, the slope, although does not significantly differ from zero, indicates that a 1h-delay in chronotype from 1st to 5th year leads to a 7min decrease (95% CI=-19-6min) in SDw. This tendency is inverted for afternoon- and evening-attending students, who increment their sleep in 11min (95% CI=1-22min) and 31min (95% CI=17-45min), respectively, for each hour their chronotype is delayed. N=259.

Supplementary Files

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