# Interplay of chronotype and school timing predicts school performance

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Most adolescents exhibit very late chronotypes and attend school early in the morning, a misalignment that can affect their health and psychological well-being. Here we examine how the interaction between the chronotype and school timing of an individual influences academic performance, studying a unique sample of 753 Argentinian students who were randomly assigned to start school in the morning (07:45), afternoon (12:40) or evening (17:20). Although chronotypes tend to align partially with class time, this effect is insufficient to fully account for the differences with school start time. We show that (1) for morning-attending students, early chronotypes perform better than late chronotypes in all school subjects, an effect that is largest for maths; (2) this effect vanishes for students who attend school in the afternoon; and (3) late chronotypes benefit from evening classes. Together, these results demonstrate that academic performance is improved when school times are better aligned with the biological rhythms of adolescents.

ost humans are active during the day and rest at night1,2. However, even when exposed to identical light-dark conditions, individuals differ in their chronotypes (that is, diurnal preferences or internal timing), ranging on a continuum between early types (larks, who wake up early in the morning) and late types (owls, who wake up later during the day)3-5. Although chronotype has a genetic basis<sup>6-8</sup>, several factors can modulate chronotype, including environmental (that is, light exposure<sup>1,9-11</sup>), biological (that is, age12-14), and social and cultural factors (that is, lifestyle<sup>15</sup>). Chronotype can be assessed by evaluating physiological<sup>16-18</sup> and behavioural rhythms<sup>19-21</sup>, but it can also be estimated using standardized questionnaires. The morningness-eveningness questionnaire (MEQ)22 produces a score that is associated with diurnal preferences. The Munich chronotype questionnaire (MCTQ)<sup>23</sup> produces a local time that reflects sleep timing. Both of these questionnaires constitute an easy, reliable and effective method to inquire about chronotypes and sleep-related variables. Although the resulting chronotype indices are not identical, their outcomes are highly correlated and reflect differences in the phase of endogenous physiological<sup>16-18,22,24,25</sup> and sleep-rest activity rhyt hms<sup>19-21,23,26</sup>. These indices are often used interchangeably<sup>9,27,28</sup> and we refer to them hereafter as chronotype.

Waking times are mostly set by work and education schedules during weekdays<sup>2</sup>. However, on free days, waking times are usually delayed to approach the endogenous tendencies of individuals<sup>21</sup> and, consistent with this, sleep duration is usually longer. This explains why the midpoint of sleep on free days, corrected by oversleep (MSFsc), is used as a measure of chronotype based on sleep timing <sup>5,12</sup> (Supplementary Fig. 1). When the endogenous biological timing (determined by the main circadian clock, located at the suprachiasmatic nuclei in the mammalian brain<sup>29</sup>) and the social timing (determined by social cues) are not correctly aligned, a chronic condition that is characteristic of modern life, known as social jetlag (SJL)<sup>2,5</sup>, is elicited. SJL is measured as the difference in sleep timing between free days and weekdays<sup>2,30</sup>. It is important to clarify that

SJL differs from sleep loss, which is the difference between the sleep duration on week days and free days (Supplementary Fig. 2).

Chronotypes become progressively delayed during development, reaching a peak of lateness during late adolescence 12-14. This biological predisposition contrasts with the most habitual school start times-adolescents all over the world attend school very early in the morning. Many adolescents therefore exhibit high SJL and short sleep duration during weekdays (that is, sleep loss)30,31. Both conditions have been associated with major health and psychological problems, such as obesity, depression, higher rates of suicide and impaired cognitive performance30,32-36 and, as this relationship predicts, delaying school start time decreases adolescents' diurnal somnolence and car-crash rates, as well as improving their wellbeing, mood and academic performance<sup>37-40</sup>. Thus, these results suggest that a better alignment between school schedules and the late chronotypes of adolescents is beneficial for their health and performance. A better alignment might result from a later school start time (as explained above) or from chronotypes becoming earlier, modulated by social cues (such as school timing).

However, studies do not usually take into account the large and intrinsic variability in the chronotype of adolescents. If school performance does indeed depend on the relationship between chronotype and schedules, then students with the latest chronotypes will perform better in late school hours, whereas students with the earlier chronotypes will perform better in the morning. Closer, but still indirect, evidence in favour of this hypothesis comes from studies showing that, in morning school hours, adolescents with early chronotypes perform better than those with late chronotypes<sup>27,41,42</sup>. However, these results are compatible with two distinct hypotheses: (1) individuals with early chronotypes always perform better than individuals with late chronotypes (chronotype effect) or (2) individuals with early chronotypes obtain higher academic performance than individuals with late chronotypes because they are evaluated at their best time of the day (synchrony effect)<sup>43-47</sup>.

#### Box 1 | Hypotheses and predictions

## Three alternative hypotheses may explain the extent to which school timing can affect the chronotypes of adolescents.

Hypothesis 1: the chronotypes of adolescents are not affected by school timing (no modulation). Adolescents will exhibit very late chronotypes, without differences among school timing tracks. Students attending school earlier will have higher levels of SJL and sleep loss (Supplementary Fig. 2a).

Hypothesis 2: the chronotypes of adolescents fully align with their school timing (full modulation). Chronotypes will vary widely with school timing (with an extent comparable to differences in school timing). Levels of SJL and sleep loss will be very low, independent of school schedules (Supplementary Fig. 2b).

Hypothesis 3: the chronotypes of adolescents only partially align with their school timing (partial modulation). Chronotypes will vary slightly among school schedules, with earlier chronotypes in the morning and later chronotypes in the evening school schedules. Because the modulation of chronotypes does not fully compensate differences in school start time, students that attend school earlier will have higher levels of SJL and sleep loss (Supplementary Fig. 2c).

As daily social cues affect circadian rhythms<sup>15</sup>, we predict that school timing will modulate the chronotypes of adolescents (reducing the misalignment between school schedules and internal timing). However, considering that the external light–dark cycle is the strongest factor that affects chronotypes<sup>1,9</sup>, we predict that the school timing modulation will only be partial, consistent with H3. Importantly, as chronotypes become later during adolescence<sup>12–14</sup>, age will modulate how school timing affects chronotype.

## Three alternative hypotheses may explain how the chronotype and the synchrony effects affect academic performance.

The interplay of late chronotypes with early school start times is supposed to be the cause<sup>27,31</sup> of unwanted sleep-associated outcomes that were previously associated with lower academic performance (for example, short sleep duration, high SJL, sleep inertia)<sup>27,48,66,86-88</sup>. Consistent with this, all of the following hypotheses assume that the chronotype is a better predictor of academic performance than sleep-related variables. Thus, we predict that models that include chronotype will be more parsimonious<sup>62</sup> than models that include SJL or sleep duration.

When school subjects are analysed separately, students with early chronotypes who attend school during the morning perform better than students with late chronotypes in maths and chemistry, but this effect is smaller<sup>48</sup> or absent<sup>49</sup> in language or geography. Outside of the classroom, evidence of synchrony effects was found in explicit memory<sup>50</sup>, priming<sup>51</sup>, executive functioning<sup>45,52,53</sup> and fluid—but not crystallized—measurements of intelligence<sup>44,46</sup>. Thus, school subjects with different cognitive requirements might be differentially affected by the synchrony and/or the chronotype effect.

To test how these two effects affect academic performance, it is necessary to evaluate grades at different times of the day, including the best time for each chronotype (that is, morning for students with earlier chronotypes, and afternoon, or even later, for students with later chronotypes). A few studies compared morning and afternoon academic performance and showed that adolescents with early chronotypes perform better than late chronotypes during the morning hours, but the opposite relationship was not found during the afternoon<sup>47,54–58</sup>. This suggests that the chronotype effect is not the only effect that affects academic performance (as students with earlier chronotypes did not perform better than students with late chronotypes during the afternoon). However, in these studies, the

Hypothesis 1: the synchrony effect completely explains variations in academic performance. School timing will reverse the relationship between grades and chronotype—in the morning, adolescents with earlier chronotypes will have better academic performance. Instead, in the evening, performance will be better for later chronotypes (Supplementary Fig. 3a).

Hypothesis 2: the chronotype effect completely explains variations in academic performance. Students with earlier chronotypes will have better grades than students with later chronotypes<sup>27,41,42</sup> in all school timings (Supplementary Fig. 3b).

Hypothesis 3: both the synchrony and the chronotype effects (combined effect) explain variations in academic performance. In the morning, individuals with earlier chronotypes will have better grades than individuals with later chronotypes. In the afternoon and in the evening, the synchrony effect will buffer the chronotype effect—the strength of the association between chronotype and grades will be weaker or even reversed. This will occur particularly for older adolescents (as chronotype becomes later with age<sup>12–14</sup>; Supplementary Fig. 3c).

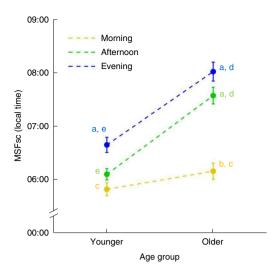
Evidence compatible with the chronotype and the synchrony effects was previously reported<sup>27,41–47,50–57</sup>. We predict that these effects are not mutually exclusive and that they, in fact, might work together to affect academic performance, consistent with H3.

Previous studies<sup>44,46,48,49</sup> suggest that the impact of the chronotype and the synchrony effect on grades depends on school subject. Some of those studies have also shown that these effects are more prominent in maths than in other subjects<sup>42,49</sup>. However, because these studies were conducted in schools with morning schedules, showing that early chronotypes perform better than late chronotypes, we cannot set expectations about whether the specificity to maths is a consequence of chronotype, synchrony effect or both.

For the reasons mentioned above, we expect that the strength of the association between students' chronotypes and grades depends on school timing, school subject and age. We therefore predict that models that include the fourfold interaction among chronotype, age, school subject and school timing are the most parsimonious to explaining the academic performance of adolescents.

students' assignment to different school timings was not random, which might mask the existence of a pure chronotype effect. It is unclear, however, whether afternoon school hours are still too early to find an advantage for students with late chronotypes (a pure synchrony effect) or whether the chronotype effect is buffered by the synchrony effect. Thus, current data show a relationship between school schedules and performance, but cannot specifically answer whether this relationship results from a chronotype, a synchrony or their combined effect (Supplementary Fig. 3).

A direct answer to this simple question requires a very specific experimental condition (in which schedules are not dependent on other social, cultural or economic factors) that is extremely rare to find in natural educational setups. Here we capitalize on an experimental sample (753 students) that enabled us to study the relationship between school performance, school timing and chronotypes (Box 1). The key aspect is that we worked with a specific public school in the city of Buenos Aires, in which—at the beginning of high school—adolescents are randomly assigned by a lottery system to morning (07:45–12:05), afternoon (12:40–17:00) or evening (17:20–21:40) school schedules. The random assignment means that it is highly unlikely that any school-timing-selection biases are



**Fig. 1** | In adolescents, chronotype (MSFsc) is related to the interaction between age and school timing. The difference in MSFsc between evening and morning school timing is 50 min in younger adolescents (06:39 versus 05:49), and it doubles to 111.6 min in older adolescents (08:01 versus 06:10). Similarly, older students who attend school in the afternoon exhibit a delay of 84.6 min in MSFsc compared with those who attend in the morning (07:34 versus 06:10). The dashed lines connecting independent age groups were added as a visual guide. Data are mean  $\pm$  s.e.m.; n = 747. The lowercase letters indicate significant differences between groups: a, compared with morning; b, compared with afternoon; c, compared with evening; d, compared with younger students; e, compared with older students. Post hoc pairwise comparisons, P < 0.006 (Bonferronicorrected P < 0.05). Data distribution is provided in Supplementary Fig. 4 and detailed information about the statistical analysis is provided in Supplementary Table 2.

introduced on the basis of previous academic achievement<sup>54</sup>, socio-economic status or chronotype preferences. Instead, in this sample, all demographic and chronotype variables are randomly assigned to each school start time, enabling us to test the effect of the interaction between school schedules and chronotype on academic performance in both younger (13–14 years of age) and older (17–18 years of age) adolescents.

#### Results

The interaction between school timing and age is associated with adolescents' chronotype and sleep. To determine whether school timing impacts daily rhythms of adolescents (Box 1), we performed a two-way analysis of variance (ANOVA), with MSFsc<sup>12</sup> as the dependent variable and school timing (morning, afternoon and evening; Supplementary Table 1) and age group (younger and older adolescents) as factors. In line with previous observations<sup>12,13</sup>, we found a main effect of age ( $F_{1,741} = 82.513$ , P < 0.0001, partial  $\eta^2 = 0.100$ , 90% confidence interval (CI) = 0.0685-0.135) showing later chronotypes for older adolescents. We also observed a main effect of school timing ( $F_{2,741}$ =45.225, P<0.0001, partial  $\eta^2 = 0.109$ , 90% CI = 0.0748-0.143), with progressively delayed chronotypes as school starts later in the day (Fig. 1, Supplementary Fig. 4, Supplementary Table 2). This result indicates that adolescents attending different school schedules during weekdays exhibit different sleep timings on free days (wake-up and sleep onset times are provided in Supplementary Table 3). Specifically, students who attend morning classes had significantly earlier chronotypes than those who attend school during the evening. This suggests that the biological time of students is better aligned to the specific social norms to which they were

assigned randomly. Similar results were obtained for MEQ score (Supplementary Results 1a).

Above and beyond those main effects, variations in chronotype through adolescence are related to school schedule (Fig. 1). This is revealed by a significant interaction of school timing and age with chronotype ( $F_{2,741} = 9.913$ , P < 0.0001, partial  $\eta^2 = 0.0261$ , 90% CI = 0.0093–0.0463). The nature of this interaction is clearly shown in Fig. 1; younger adolescents show a small effect of school timing on chronotype. However, this effect is amplified in older students, suggesting that chronotypes are modulated by social cues and that this is a slow process that builds up during adolescence.

To evaluate whether the adjustment in chronotype fully or partially compensates for its misalignment with school timing (Box 1), we tested whether levels of SJL and sleep duration also depend on the interaction between school timing and age. We found that SJL is affected by the interaction between the two factors ( $F_{2.741} = 6.590$ , P = 0.001, partial  $\eta^2 = 0.0175$ , 90% CI = 0.0042-0.0346; Supplementary Figs. 5 and 6, Supplementary Table 4, Supplementary Results 1b). Morning school hours are associated with high SJL (close to 4h in both age groups). Sleep duration was also affected by the interaction between school timing and age  $(F_{2.741} = 3.246, P = 0.039, partial \eta^2 = 0.009, 90\% CI = 0.0004 - 0.0222;$ Supplementary Figs. 7 and 8, Supplementary Table 5, Supplementary Results 1c). The short sleep duration—especially for students who attend school in the morning—is only partially overcome by compensating for nocturnal sleep with naps (Supplementary Figs. 7b, 9 and 10, Supplementary Table 6).

On the whole, our findings indicate that the morning schedule starts too early relative to adolescents' internal rhythms. Even though adolescents' chronotype partially aligns with morning school timing, this compensation is insufficient and, even considering naps, students who attend school in the morning do not reach the minimum 8 h of sleep that is recommended for adolescents<sup>59-61</sup>. On the contrary, the evening schedule starts sufficiently late for all students. In between, the afternoon schedule (which starts at 12:40) still poses a considerably sleep challenge for older adolescents.

The effect of the synchrony between chronotype and school timing on academic performance depends on school subject and age. To test whether chronotype is a better predictor of academic performance than sleep-related variables, and how school timing, school subject and age modulate the strength of the association between chronotype (or sleep-related variables) and grades (Box 1), we ran a set of linear mixed-effects models using academic performance as the dependent variable. These models include a chronotype or a sleep-related variable that interacts with other predictors (school timing, school subject and age; Supplementary Table 7).

The most parsimonious model was selected on the basis of Akaike's information criteria (lower AIC<sup>62</sup>). As we hypothesized (Box 1), it included the fourfold interaction among chronotype, age, school subject and school timing, controlled by gender (MSFsc model; Table 1, Supplementary Table 8;  $F_{4.676}$  = 3.390, P = 0.009, partial  $\eta^2$  = 0.020, 90% CI = 0.003 – 0.035). The first implication of this result is that chronotype better predicts academic performance than sleep-related variables. This result also indicates that academic performance is affected by the interaction between chronotype and school timing (that is, the synchrony effect), depending on age and school subject.

Chronotype was significantly correlated with academic performance (b=-0.157, 95% CI=-0.255 to -0.059, t=-3.157), indicating that, overall, individuals with later chronotypes obtain lower grades than individuals with earlier chronotypes. Specifically, a chronotype of 1 h later with respect to the average (MSFsc=06:37) was associated with a decrease in maths grades of 0.315 points and with a decrease of 0.157 grade points in all of the school subjects that were neither maths nor language (referred to as other subjects; Table 2).

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Table 1   ANOVA results for the most parsimonious model explaining academic performance (MSFsc model)						
	Sum Sq	d.f.	F	P	Partial $\eta^2$	90% CI
MSFsc	88.530	1	33.910	<0.0001	0.048	0.025-0.076
School timing	10.480	2	2.010	0.146	0.006	0-0.017
School subject	2,707.210	2	518.520	< 0.0001	0.605	0.570-0.635
Age	18.250	1	6.990	0.011	0.010	0.001-0.026
Gender	78.410	1	30.040	<0.0001	0.043	0.021-0.070
MSFsc:school timing	25.820	2	4.950	0.007	0.014	0.002-0.031
MSFsc:school subject	18.440	2	3.530	0.029	0.010	0.001-0.025
School timing:school subject	109.960	4	10.530	< 0.0001	0.059	0.029-0.085
MSFsc:age	1.530	1	0.590	0.444	0.001	0-0.008
School timing:age	6.960	2	1.330	0.273	0.004	0-0.013
School subject:age	290.630	2	55.670	< 0.0001	0.141	0.102-0.180
MSFsc:school timing:school subject	75.460	4	7.230	< 0.0001	0.041	0.016-0.063
MSFsc:school timing:age	12.210	2	2.340	0.097	0.007	0-0.019
MSFsc:school subject:age	4.130	2	0.790	0.453	0.002	0-0.010
School timing:school subject:age	32.920	4	3.150	0.013	0.018	0.002-0.033
MSFsc:school timing:school subject:age	35.440	4	3.390	0.009	0.020	0.003-0.035

Significant interactions imply changes in the slope and/or the intercept of the regression lines between MSFsc and grades. Specifically, interactions between MSFsc and other predictor(s) are associated with changes in the slope. Interactions among other predictors are associated with changes in the intercept. Retrospective power for the four-way interaction in this model was 90% (approximated using bootstrapping  $^{84,85}$ ); n = 712. Sum Sq, sum of squares.

Figure 2 and Table 2 show the slopes of the association between chronotype and grades for the different levels of the predictors. The slopes are negative for all of the students in the morning (that is, individuals with early chronotypes obtained higher grades than individuals with late chronotypes, as expected due to chronotype and/or synchrony effects; younger and language:  $\beta$ =-0.177, 95% CI=-0.305 to -0.049, t=-2.712; younger and maths:  $\beta$ =-0.315, 95% CI=-0.443 to -0.187, t=-4.825; older and language:  $\beta$ =-0.166, 95% CI=-0.301 to -0.032, t=-2.421, older and maths:  $\beta$ =-0.338, 95% CI=-0.473 to -0.202, t=-4.877), whereas, in the afternoon and in the evening, the slopes reveal a much wider pattern with age, school timing and school subject.

For younger students attending in the afternoon, grades were not associated with chronotype, as evidenced by the slopes being close to zero (no chronotype differences; language:  $\beta$ =0.019, 95% CI=-0.117-0.155, t=0.278; maths:  $\beta$ =-0.066, 95% CI=-0.202-0.070, t=-0.952), supporting the synchrony effect. However, for younger students attending school during the evening, students with early chronotypes also performed better than students with late chronotypes (slopes were negative; language:  $\beta$ =-0.194, 95% CI=-0.315 to -0.074, t=-3.167; maths:  $\beta$ =-0.166, 95% CI=-0.286 to -0.046, t=-2.702), supporting the chronotype effect prediction. (Fig. 2a).

For older students, results differ (Fig. 2b). In the afternoon, maths grades were not associated with chronotype ( $\beta$ =-0.019, 95% CI=-0.149-0.110, t=0.289), but students with early chronotypes performed better than students with late chronotypes in language ( $\beta$ =-0.142, 95% CI=-0.271-0.012, t=-2.143). In the evening, the slopes between grades and chronotype did not differ from zero for both maths ( $\beta$ =-0.074, 95% CI=-0.209-0.061, t=-1.075) and language ( $\beta$ =0.113, 95% CI=-0.022-0.248, t=1.637). However, the association between language grades and chronotype for older evening-attending students is more positive and significantly higher than all of the other relevant comparisons (versus their maths grades: t=2.697, Cohen's d=0.116, 95% CI=0.032-0.200; versus their younger peers: t=3.329, d=0.190, 95% CI=0.078-0.302; versus their morning-attending peers: t=2.869, d=0.173, 95% CI=0.055-0.291; and versus their

afternoon-attending peers: t=2.664, d=0.157, 95% CI=0.042–0.273; Supplementary Table 9). This last result is consistent with the synchrony effect—individuals with later chronotypes tend to have higher grades than individuals with earlier chronotypes.

All of these results suggest that the synchrony effect acts differently according to age and school subject. However, overall, performance is worse for later chronotypes, which means that a chronotype effect also affects academic performance. Taken together, this pattern reveals that grades are affected by a complex combined effect, with a differential impact of chronotype and synchrony effects depending on school subjects and adolescents' age.

#### The extent to which chronotype influences school performance.

In this school, physical education is conducted outside of typical hours (Supplementary Table 1). This enabled us to evaluate the association between chronotype and academic performance when students who regularly attend school in the morning, afternoon or evening were evaluated at another time of the day. If the synchrony effect affects academic performance, students who attend physical education very early in the morning (independent on their regular school timing) will have the stronger association between physical education grades and chronotype, and this synchrony effect will strongly impact academic performance of older students. Consistent with this, the most parsimonious model explaining physical education grades included the three-way interaction, with chronotype interacting with school timing and age (Supplementary Tables 10–12, Supplementary Results 2;  $F_{2.700} = 5.363$ , P = 0.005, partial  $\eta^2 = 0.015$ , 90% CI = 0.003-0.032). A chronotype of 1h later was associated with a decrease of 0.123 grade points in physical education (Supplementary Tables 12 and 13; b = -0.014, 95% CI = -0.009 - 0.036, t = 1.211). Although no statistically significant differences were found for younger students (Supplementary Table 14), the slope of the regression line between physical education grades and chronotype of older students was significantly steeper among the students who attend physical education during the earliest schedule (Supplementary Figs. 13 and 14, Supplementary Tables 13-15). In line with the previous section, these results indicate that a combined effect also affects physical education grades.

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Age group	School subject	School timing	later MSFsc	Intercept	Slope	95% CI	τ
Younger	Other subjects	Morning	7.230	7.387	-0.157	-0.254 to -0.060	-3.157
Younger	Other subjects	Afternoon	7.335	7.455	-0.120	-0.223 to -0.017	-2.273
Younger	Other subjects	Evening	7.345	7.440	-0.095	-0.187 to -0.003	-2.025
Younger	Language	Morning	6.271	6.448	-0.177	-0.305 to -0.049	-2.712
Younger	Language	Afternoon	6.922	6.903	0.019	-0.117 to 0.155	0.278
Younger	Language	Evening	6.949	7.143	-0.194	-0.315 to -0.074	-3.167
Younger	Maths	Morning	5.936	6.251	-0.315	-0.443 to -0.187	-4.825
Younger	Maths	Afternoon	6.358	6.424	-0.066	-0.202 to 0.070	-0.952
Younger	Maths	Evening	5.843	6.009	-0.166	-0.286 to -0.046	-2.702
Older	Other subjects	Morning	7.326	7.491	-0.165	-0.267 to -0.063	-3.184
Older	Other subjects	Afternoon	7.353	7.524	-0.170	-0.268 to -0.072	-3.408
Older	Other subjects	Evening	7.218	7.234	-0.016	-0.118 to 0.086	-0.302
Older	Language	Morning	7.253	7.419	-0.166	-0.301 to -0.032	-2.421
Older	Language	Afternoon	7.781	7.923	-0.142	-0.271 to -0.012	-2.143
Older	Language	Evening	7.567	7.454	0.113	-0.022 to 0.248	1.637
Older	Maths	Morning	6.027	6.364	-0.338	-0.473 to -0.202	-4.877
Older	Maths	Afternoon	6.252	6.271	-0.019	-0.149 to 0.110	-0.289
Older	Maths	Evening	5.927	6.001	-0.074	-0.209 to 0.061	-1.075

To obtain a more natural interpretation of the model's estimates, MSFsc was included relative to its global mean (M = 06:37); n = 712. Each intercept results from the sum of the corresponding coefficients and indicates the predicted grade on each group of conditions for a female student with an average chronotype (Supplementary Table 8). Each slope indicates the predicted change in grades for an MSFsc of 1h later.

#### Discussion

Here we studied the extent that school schedules affect chronotypes and how this interaction affects sleep and grades among adolescents of different ages. Our results demonstrate that a better alignment between chronotypes and school timing is associated with higher academic performance in adolescents randomly assigned to different school schedules, and that the extent of improvement is dependent on school subject and age. As shown previously<sup>27,41,42</sup>, we found that students with earlier chronotypes attending school in the morning perform better than students with later chronotypes. Here we showed that this effect results from a combination of a chronotype and a synchrony effect. In fact, the chronotype effect is greatly reduced in the evening school timing, when it almost reverses (students with later chronotypes perform slightly better in language than the students with earlier chronotypes). These results suggest that students with late chronotypes can perform better than students with early chronotypes at school. Our evidence on sleep patterns and chronotypes also explains why it has been so difficult to observe this result previously. We show that even afternoon school timing (starting close to noon) is associated with high levels of SJL and short sleep duration in older adolescents and, therefore—contrary to simple intuitions—the afternoon might not be the best time for students with late chronotypes.

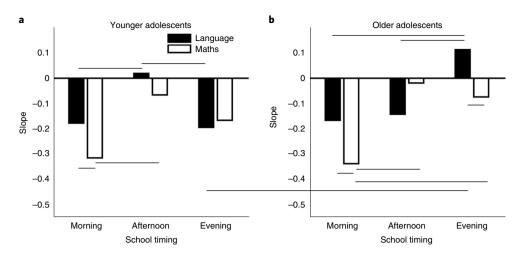
We also showed that school schedules are associated with differences in adolescents' chronotypes and sleep habits. This result shows that adolescents' chronotypes partially, but not fully, align with school schedules. Students attending school in the morning have earlier chronotypes than students attending school in the afternoon or in the evening, but partially compensate their short nocturnal sleep by substantially increasing nap time. However, even by combining these effects (partial adaptation of chronotypes and diurnal naps) adolescents who attend school in the morning are unable to compensate for the difference between their endogenous time and the early school start time.

As expected, age was associated with changes in chronotypes, with older adolescents exhibiting later chronotypes<sup>12-14</sup>. However, these changes were only found in the afternoon and in the evening. Unexpectedly, there were no age differences in morning-attending students, suggesting that older adolescents further adapt their chronotype to the morning schedule. Future research is necessary to address the longitudinal changes in chronotype among adolescents who attend school at different times.

A comparison with previous studies 12-14,30,55-57,63,64 shows that this Argentinian sample includes students with later chronotypes than those measured in other countries, but similar school start times to those in the rest of the world 63. Later chronotypes were expected because, in the city of Buenos Aires, dinner time is typically around 21:00 and late activities are much more frequent than in Europe or the United States; school schedule will therefore probably and strongly affect adolescents' sleep. This can also explain why even adolescents who attend school in the evening have more than 1.5h of SJL—during weekdays they go to sleep later than 00:30, although earlier than on free days. However, on weekdays, they typically wake up earlier than expected, on the basis of school timing, to study or to do other activities before going to school. This variability is beyond the scope of the present study.

If one were to reflect on the practical consequences of this and other similar studies, then it is important to understand that there may be some general principles, such as the effect of synchrony. How exactly this synchrony is achieved may depend on cultural idiosyncrasies of each society. Indeed, a previous study in adolescents from Buenos Aires found high daytime somnolence, which correlated with short sleep and poor academic performance<sup>65</sup>.

Students who attend school in the morning have late chronotypes compared with those from other countries<sup>12,13</sup>. Nevertheless, their chronotypes are earlier compared with their peers who attend school later in the day. School timing influences chronotypes but it is not sufficient to eliminate SJL or to reach the recommended levels



**Fig. 2** | Slopes of the regression lines between chronotype (MSFsc) and grades depend on school timing, age and school subject. **a**, Slopes for younger students. **b**, Slopes for older students. A null slope implies no association. A negative slope indicates higher grades for earlier chronotypes. A positive slope indicates higher grades for later chronotypes; raw values are provided in Table 2; n = 712. The lines indicate the significant pairwise comparisons. The theoretical interpretation is provided in Supplementary Fig. 3, data distribution is provided in Supplementary Figs. 11 and 12, and detailed information about the statistical analysis is provided in Supplementary Table 9.

of sleep duration (8–10 h)<sup>59–61</sup> for those students attending morning classes. On average, they sleep for less than 6.75 h on weekday nights (93.5% of students sleep less than the minimum recommended level). Even including the average nap time, morning-attending students sleep less than 8 h a day. This sample of students is therefore exposed to chronic sleep deprivation, a condition that is elsewhere associated with daytime sleepiness, reduced health, and emotional and cognitive impairment<sup>32,33</sup>. Furthermore, morning-attending students experience almost 4 h of SJL (92.28% of students exhibit more than 2 h). High levels of SJL in adolescents have been associated with lower cognitive performance, risky behaviour, obesity and depression<sup>34,35,66</sup>. As a consequence, morning school hours constitute an extremely adverse environment for these students.

Even though morning-attending students exhibit critical levels of SJL and sleep duration on weekdays, they performed just as well as students attending at other school hours. One possible compensatory mechanism that might be balancing the detrimental effects on cognitive abilities is the after-learning effect of naps: students attending the morning school schedule can only nap after school, and this closeness could have the additional benefit of sleeping after learning, which is strongly associated with better memory consolidation<sup>67–69</sup>. Naps could also be adding crucial sleep time<sup>70</sup>. Alternatively, morning-attending students could be developing other abilities that enable them to compensate for the sleep and misalignment handicap (such as higher conscientiousness<sup>71,72</sup>).

Our results show that the association between chronotype and academic performance for morning-attending students was always such that students with later chronotypes were associated with worse performance. This result is in agreement with previous findings<sup>27,42</sup> and consistent (albeit not conclusive) with the hypotheses of a synchrony effect. Interestingly, maths grades were more strongly influenced by chronotype, with larger differences in performance between individuals with late and early chronotypes. This last result is also in agreement with previous observations, in which the magnitude of the chronotype effect on academic performance in school subjects such as maths was stronger<sup>48,49</sup>, and also with a synchrony effect that was found between chronotypes and time of the day on cognitive tasks related to fluid intelligence but not those related to crystallized intelligence<sup>44,46</sup>.

However, as argued above, results from the morning school schedule cannot discriminate between a chronotype effect (that is,

individuals with early chronotypes could simply perform better), a synchrony effect or their combined effect. The critical test to discriminate among these hypotheses comes from analysing the performance of adolescents who attend school in conditions in which students with later chronotypes would be better aligned with school timing than students with earlier chronotypes. Our quantitative study of chronotypes shows that this happens only for the evening school timing and, consistent with the synchrony hypothesis, we observed that, during the evening school timing, students with late chronotypes perform slightly better in language than students with early chronotypes (Fig. 2). In this case, the synchrony effect affects academic performance. However, a chronotype effect is also present because the effect of chronotype on grades depends on the school subject and on the age of the adolescents. The fact that younger students have earlier chronotypes than older students can explain why the evening school hours do not revert the chronotype effect in young people. It is possible that the evening is too late for younger students; in other words, the best time for students with late chronotypes is earlier in younger students compared with older students. Together, our results suggest that the combined effect is affecting academic performance of adolescents, with the synchrony effect buffering the chronotype effect.

The extent of the combined effect depends on age, school subject and time of the day. It is particularly important that the synchrony effect favours late chronotypes in language for 17–18-year-old adolescents who attend school in the evening. Future studies are certainly required to understand the cognitive mechanisms that underlie these differences<sup>44,71–73</sup>. Another question for future research is to determine the relative contribution of learning and evaluation times to the effect of chronotype on academic performance.

This research has several limitations: it is based on regression analyses (we could not establish causality between chronotypes/ sleep habits and academic performance), and circadian preferences and sleep habits were assessed using questionnaires (which are self-reported and subjective). Although a direct bias is extremely unlikely because students were blind to the experimental hypotheses when they completed the questionnaires, actigraphy would be a better method to assess these variables. When analysing the impact of school timing on chronotype, sleep duration and SJL, we did not include gender as a factor and, therefore, our findings might not generalize over gender. Another of the limitations is that the set of

predictors did not include other variables to which we did not have access that could potentially affect chronotype or sleep habits on academic performance, such as school attendance, motivation, psychosocial environment and socioeconomic status. Related to this, this school is very prestigious, with an entry examination, which might render our sample biased in favour of a high-performance group of students. Not taking these factors into account might result in an overestimation of the studied associations. Another limitation is the cross-sectional design: even though school schedule is assigned by lots, a longitudinal analysis might confirm that chronotypes adapt to school timings. Another limitation lies in the fact that we did not ask about medication or illnesses and, therefore, we cannot exclude differences in the proportion of students taking medication or having illnesses among school timings. Moreover, these students had physical education of outside typical hours twice a week and, therefore, the school schedules are not completely morning, afternoon or evening. The use of grades as the academic performance measurement also has several constraints—they are not strictly objective owing to the absence of standard exams and different grading depending on school subjects or teachers. However, given the high number of courses we tested and the broad teacher distribution across school timings, it is highly improbable that these effects may introduce biases into our results. Importantly, even when present, those constraints would account for differences between courses or school timings and would not explain the differences in the association that we found between chronotypes and grades at different school timings. Finally, the lack of baseline information about the chronotypes of students does not allow us to completely rule out the possibility that—even though highly improbable—the lottery assignment of school timings might have favoured students with late (or early) chronotypes assigning them to late (or early) evening school schedules, even with the random assignment.

This research also has specific strengths. First, we worked with a school that has three timing schedules that are atypically widely spread throughout the day and include the evening. Second, our study includes adolescents of two different age groups, therefore enabling us to assess the effect that the interaction between chronotype and time of the day has on academic performance at different ages. Third, we had access to nap information and could therefore address the full extent of sleep and not only nocturnal sleep. Finally, and importantly, students were randomly assigned to each schedule. The high number of participants and the random assignment enabled us to assume that there should be no differences in chronotypes before beginning school and, therefore, that chronotypes are affected by school schedule. It is worth noting that students were randomly assigned to very different school schedules. A previous cross-sectional study of college students randomly assigned to different morning school start times (07:00, 07:30 and 07:50) found a positive effect on academic performance associated with a later school start time<sup>74</sup>.

Several practical implications arise from this work; however, these may require translational studies to shed light on their potential applications. First, performance in all of the school subjects was higher for students with earlier chronotypes during the morning; however, the effect on maths performance was stronger than the effect on language. Thus, a practical implication of our study is that the school schedule should be adapted to teach maths later during the morning, even in the first year of high school. This modification could benefit late chronotypes who attend school in the morning. Second, academic performance is influenced by the interaction between chronotype and age. School start times could therefore be progressively delayed throughout adolescence. This is probably difficult to achieve, but delaying school start times only for older adolescents could be adopted as an intermediate solution that favours the most vulnerable population of students. Previous studies have found benefits for the sleep and academic performance of adolescents after delaying the school start time<sup>37-40</sup>, but these effects should be evaluated in the long term to assess whether adolescents adapt after a while on the delayed schedule<sup>15,39,75</sup>. Other interventions could be evaluated, such as light exposure during the morning, automatic regulation of blue light levels on LED-screen devices and educational interventions<sup>76-79</sup>.

We emphasize that more evidence is required before making strong claims on the practical implications of this study. However, our results indicate that the importance of biological variability in daily rhythms for academic performance may have been underestimated. We are aware that a full alignment of individual circadian preferences and school timing is completely impractical. However, our data suggest that assigning students to specific school timings on the basis of their chronotypes, or organizing the order of school subjects according to the age of students, may be among other simple procedures that might improve school performance by understanding the best time for each student.

#### Methods

Ethical approval. The study was approved by the institutional Ethical Committee of the Universidad Nacional de Quilmes (Verdict #4/2017) and by the head of the school. The study was conducted according to ethical recommendations for human chronobiological research On the basis of the Argentinian national regulations, our study was not invasive of the integrity of the participants, and it was performed during regular school hours. Students provided oral informed consent to participate.

**Participants.** This study was performed at a local secondary school in the City of Buenos Aires, Argentina (34° 60′ S, 58° 38′ W). All of the students of the first and fifth year who attended school on the day of data collection participated in the study. The attendance percentage was similar between school timings (morning, 96.76%; afternoon, 97.72%; evening, 96.62%) and no student refused to participate. From the 767 students who completed the questionnaire, 753 students were included in at least one of the analyses of the study. The exclusion criterion was incomplete information. Each age level was homogeneous for age (first year (younger adolescents), n = 436, aged (mean  $\pm$  s.d.) 13.47  $\pm$  0.34 years; fifth year (older adolescents), n = 317, aged 17.51  $\pm$  0.38 years) and gender distribution (first year, 48.40% females; fifth year, 47.63% females). Teachers are not randomly distributed across school timings but—given the number of courses (n = 30), the high number of teachers (n = 130) and their broad distribution across courses—no systematic difference in teaching quality and exigency can be assumed.

**Procedure.** We obtained school grades of all of the students attending the first and the last (fifth) year of this secondary school. Students from a total of 30 classrooms participated in the study. These spanned two levels—15 classes belonged to the first year and the other 15 belonged to the fifth year of the school, which corresponds to all classrooms of first and fifth year from different school hours. Descriptive features of the participants are included in Supplementary Table 15.

A crucial aspect of our experimental setup is that, in this school, the school timing is set by a lottery system. At 12–13 years old, just before the beginning of their first high school year, students from this secondary school are assigned by lots to one of three school timing or school hours (morning, 07:45–12:05; afternoon, 12:40–17:00; evening, 17:20–21:40). Students cannot change the assigned school timing unless they already have brothers or sisters in the school hours they prefer to attend (which in turn were also originally assigned randomly to a given school timing), and they remain in the assigned school timing until the end of high school (fifth year, 17–18 years old). In each year and school timing, students are distributed into five classrooms. For each school timing, the academic schedule is organized into typical or outside-of-typical school hours. Most subjects are taught and examined during the typical hours of each school timing, but physical education is given twice a week and outside the expected hours for that school timing (the school schedule for each school timing is provided in Supplementary Table 1).

In June 2015, during the typical hours of each school timing, students filled Spanish versions of the MEQ<sup>22,81</sup>, the MCTQ<sup>23</sup> and demographic information (date of birth and self-defined gender). The MEQ comprises questions that relate to diurnal preferences and results on a MEQ score of between 16 and 86 points, where low values are associated with late (owl) chronotypes and high values are associated with early (lark) chronotypes<sup>22</sup>. MCTQ includes questions about sleep habits and results in a time point (MSFsc) where low values indicate early chronotypes and high values indicate late chronotypes<sup>23</sup>. Data collection and analysis were not performed blind to the conditions of the experiments.

**Measurements.** For each student, we obtained two chronotype indices: the MEQ score and the sleep-corrected midpoint of sleep time on free days (MSFsc, from MCTQ). Internal consistency of MEQ was acceptable (Cronbach's alpha = 0.7028).

The midpoint of sleep on free days (MSF) is a time of day that is calculated as the halfway point between sleep onset and sleep offset, and is then corrected by the confounder sleep loss (MSFsc = MSF – 0.5(SDf – (5 × SDw + 2 × SDf)/7))\(^{12}\) where SDf is sleep duration on free days and SDw is sleep duration on weekdays. We also obtained, from the MCTQ, the following sleep-related variables: nocturnal sleep duration on school or weekdays (SDw) and sleep duration on free days (SDf), total sleep duration on weekdays (tSDw) and on free days (tSDf) (total sleep duration included both nocturnal and nap sleep duration), and SJL (absolute difference between MSF and midpoint of sleep on weekdays; Supplementary Fig. 1). Sleep duration variables enabled us to evaluate how many hours adolescents sleep during week and free days, SJL is a measurement of the discrepancy in sleep timing between week and free days.

Not all of the students have all of the variables. Missing values occurred when a variable could not be calculated because of incomplete information (that is, a student might have a value of MEQ score but not MSFsc if they did not complete all of the MCTQ questions). Missing data were omitted from the analyses.

As usual, in the Argentinian secondary school system, different school subjects might be imparted in first and fifth year. Only three school subjects are common for both ages and are dictated at similar times of the day at this high school, and those were the only subjects that we assessed in this study: language (Spanish), maths and physical education. Both language and maths were dictated during typical hours; physical education was taught outside of typical hours. Other non-shared subjects dictated during typical hours were grouped as 'other' subjects.

All grades were obtained from the school's registration system. Each student is graded four different times for each school subject: two general grades (which are decided by the teacher after the student performance during classes and small exams), and two integrative grades (which come from two comprehensive exams that take place at specific periods of the year). To pass a school subject, grades have to fulfil two conditions: (1) each integrative exam requires a minimum grade of 4, and (2) the total sum of the four grades must be at least 26.

**Statistical analysis.** All statistical analyses were performed using the R system for statistical computing (v.3.4.1; R Core Team, 2017), the Real Statistics resource pack (v.5.11) or SPSS 20 (IBM).

We used ANOVA to perform statistical group comparisons. Kolmogorov–Smirnov tests were used to check normality. We used Student's t-tests 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). Error bars in all figures represent s.e.m.

Two-way ANOVA were performed to determine whether school timing (morning, afternoon or evening) and age group (younger and older) are related to MSFsc, MEQ and SJL. The association of type of day of the week (withinsubjects factor, week or free days) with the interaction of school timing (morning, afternoon or evening) and age group (younger and older) on sleep duration during free and weekdays and on total sleep duration was tested using three-factor ANOVA.

Nap occurrence and duration were calculated as the percentage of participants who do nap and, of those who nap, the amount of time that they nap, respectively. We report two-tailed P values calculated using Fisher's exact tests.

To test the effect of chronotype and sleep-related variables on academic performance, we performed linear mixed-effects models, including different sets of predictors (and interactions among them). All of the models included a chronotype or sleep-related variable, school timing (morning, afternoon, evening), age group (younger, older), school subject (other subjects, language, maths) as explanatory variables. Gender (female, male) was included as a fixed factor, without testing its interactions with the other predictors. In all cases, two factors, type of grade and student nested within classroom, were included as random factors. Model selection on the basis of AIC $^{62}$  was performed to select the best combination (fit) of independent variables explaining the variation in school grades. The most parsimonious model was defined as the model with the lowest AIC value $^{82}$ . The estimates of the model are the  $\beta$  coefficients for each effect. Comparison of  $\beta$  values for the interactions among different levels of predictors was assessed by changing the reference levels to obtain the t values for all comparison of interest, using the emmeans-package $^{83}$ .

There is no clear definition of d.f. for linear mixed-effects models and, therefore, precise P values cannot be estimated. In general, however, given the large number of values and subjects and the comparatively small number of fixed and random effects estimated, the t-distribution is equivalent to the normal distribution for all practical purposes (that is, the contribution of the d.f. to the test statistic is negligible). Our criterion for referring to an effect as significant is t < -2 or t > 2 (t = mean/s.e.).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

#### Data availability

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

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#### **Author contributions**

Conceptualization: A.P.G., M.S., D.A.G. and M.J.L. Data collection: A.P.G., G.B. and M.J.L. Data analysis: A.P.G. and M.J.L. Interpretation: A.P.G., M.S., G.B., D.A.G. and M.J.L. Writing: 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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## **Reporting Summary**

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Statistics						
For all statistical analys	ses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.					
n/a Confirmed						
☐ ☐ The exact sam	nple size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement					
A statement of	on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly					
The statistical Only common to	The statistical test(s) used AND whether they are one- or two-sided  Only common tests should be described solely by name; describe more complex techniques in the Methods section.					
A description	of all covariates tested					
A description	of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons					
A full descript AND variation	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)					
For null hypot	thesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted is exact values whenever suitable.					
For Bayesian a	analysis, information on the choice of priors and Markov chain Monte Carlo settings					
For hierarchic	cal and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
Estimates of e	effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated					
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.					
Software and o	code					
Policy information abo	ut <u>availability of computer code</u>					
Data collection	No software was used					
Data analysis	Data were analyzed using Matlab R2015a (Mathworks), SPSS 20.0.0 and R version 3.4.1					
For manuscripts utilizing cust	om algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.					
Data						
Policy information abo	ut <u>availability of data</u>					
•	include a data availability statement. This statement should provide the following information, where applicable:					
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The data that supports th	ne findings of this study are available from the corresponding author upon request					
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Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences					

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## Behavioural & social sciences study design

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II studies must disclo	ose on these points even when th	e disclosure is negative.			
Study description	Quantitative experimental, cross-sectional study.				
Research sample	A total of 753 students were included in the study. Each age level was homogeneous for age (first year -younger adolescents- (n=436): M=13.47, SD=0.34; fifth year -older adolescents- (n=317): M=17.51, SD=0.38) and gender distribution (first year: 48.40% females; fifth year: 47.63% females).				
Sampling strategy	Secondary school students from a total of 30 classrooms participated in the study. These spanned two levels: 15 classes belonged to the first year and the other 15, to the fifth year of the school, which corresponds to all classrooms of first and fifth year from different school hours.				
Data collection	In June 2015, during the typical hours of each school timing, students filled Spanish versions of the Morningness–Eveningness Questionnaire (MEQ), the Munich chronotype Questionnaire (MCTQ) and demographic information (birth date and self-defined gender) using pen and paper.  All grades were obtained from the school's registration system.				
Timing	Data collection was conducted in June 2015.				
Data exclusions	From the 767 students who completed the questionnaire, 753 students were included at least in one of the analyses of the study. The exclusion criterion was incomplete information.				
Non-participation	No participants dropped out.				
Randomization	A crucial aspect of our experimental setup is that in this school, the school timing is set by a lottery system. At 12-13 y.o., just before the beginning of their first high school year, students from this secondary school are assigned by lots to one of three school timing or school hours (Morning: 07:45-12:05 am; Afternoon: 12:40-05:00pm; Evening: 05:20-09:40pm). Students cannot change the assigned school timing except if they already have brothers or sisters in the school hours they prefer to attend (which in turn were also originally assigned randomly to a given school timing), and they remain in the assigned school timing until the end of high school, fifth year, 17-18 y.o.				
Reporting	for specific ma	aterials, systems and methods			
		aterials, experimental systems and methods used in many studies. Here, indicate whether each material, not sure if a list item applies to your research, read the appropriate section before selecting a response.			
Materials & expe	rimental systems	Methods			
/a Involved in the study		n/a Involved in the study			

IVIa	teriais & experimental systems	IVIE	tnoas
n/a	Involved in the study	n/a	Involved in the study
$\boxtimes$	Antibodies	$\boxtimes$	ChIP-seq
$\boxtimes$	Eukaryotic cell lines	$\boxtimes$	Flow cytometry
$\boxtimes$	Palaeontology	$\boxtimes$	MRI-based neuroimaging
$\boxtimes$	Animals and other organisms		
	Human research participants		
$\boxtimes$	Clinical data		

### Human research participants

folicy information about	studies involving numan	research participants
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Population characteristics See above All first and fifth year students that attended school during the data collection process were recruited. Recruitment Ethical Committee of the Universidad Nacional de Quilmes Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.