

# Multiple positive outcomes of a later school starting time for adolescents.

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## Abstract

**Background and objectives:** Adolescents are chronically sleep-deprived. Early school start time is a major promoting factor of adolescent's sleep deprivation. We evaluate the effect of delaying the school start time (DSST) by one hour during one week on sleep, sleepiness, cognition, and emotion using within-subject design.

**Methods:** This interventional study enrolled 48 adolescents. Sleep timing and duration was derived from 18 days of actigraphy recording, before, during and after DSST. Baseline, interventional and post-interventional evaluation of sleep quality, mood, and cognition was performed using standard questionnaires. Repeated measures ANOVA was performed to test variables between the weeks of the study.

**Results:** A total of 38 students (age mean  $16 \pm 0.81$  years old) concluded all study steps. Sleep duration increased during the intervention week (A= $7:03 \pm 0:41$ ; B= $7:35 \pm 0:48$ ; C= $6:46 \pm 0:48$ ;  $p < .01$ ), due the delaying waking up time (A= $6:54 \pm 0:12$ ; B= $7:42 \pm 0:30$ ; C= $6:46 \pm 0:15$ ;  $p < .01$ ) and no changes in bedtime (A= $23:18 \pm 0:42$ ; B= $23:29 \pm 0:36$ ; C= $23:28 \pm 0:41$ ;  $p = 0.27$ ). DSST also reduced somnolence measured by two independent questionnaires (KSS  $p < 0.01$ ; ESS  $p < 0.01$ ) and improved several aspects of mood measured by POMS and HADS Depression subscale ( $p = 0.004$ ). Improvements in the N-Back test were found, but no effects on free-recall were obtained.

**Conclusions:** Adolescents slept longer during DSST and did not change sleep onset. Starting school later was effective in improving multiple aspects from sleep patterns, subjective sleepiness, emotion and cognition. We add compelling evidence to the beneficial effects of DSST and this practice must be considered by the educational sector.

Keywords: Adolescent; School Start Time; Sleep; Sleepiness; Emotion

## Introduction

The circadian regulation of sleep changes significantly during adolescence<sup>1</sup>. Human response to light also varies during adolescence. It has been reported increased sensitivity to light for older teenagers in comparison to young ones<sup>2</sup>. Additionally, modifications in the homeostatic process that regulate sleep have been reported in adolescents<sup>3</sup>. As a consequence, adolescents experience what is known as the adolescent's sleep-phase delay, meaning that bedtime and wake up time will be delayed during this stage of development. This bioregulatory factors jointly with bioregulatory, psychosocial, and social pressures contribute to a chronically sleep-deprived adolescence<sup>4</sup>. This multifactorial organization is summarized by the Perfect Storm Model proposed by Carskadon in 2011<sup>5</sup>. According to the model, early school start time is the primary social factor forcing adolescents to an early wake-up time. Consequently, schools are not timely adjusted to adolescent's physiology resulting in sleep deprivation. Insufficient sleep in adolescents is a public health concern<sup>6</sup> being reported in several countries<sup>7-11</sup>.

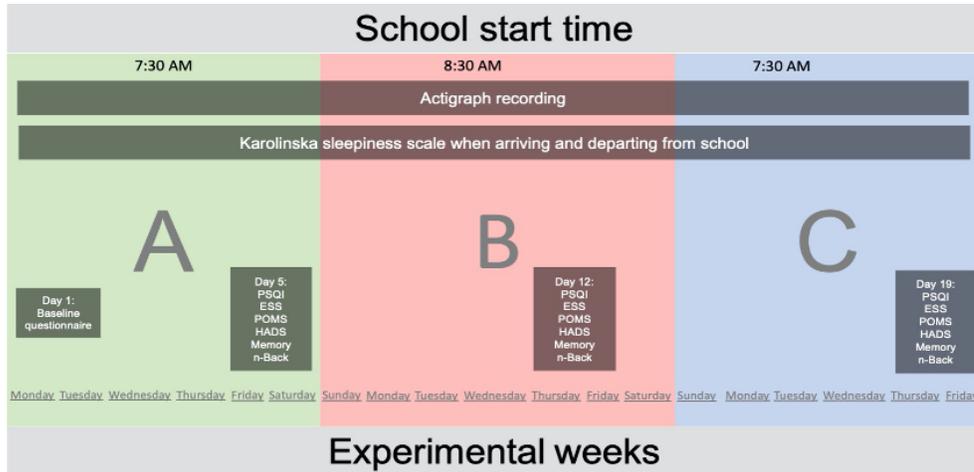
Strategies to tackle adolescent sleep deprivation have been proposed, such as educational programs to raise awareness and teach teenagers about sleep, sleep physiology, and sleep hygiene. Essentially, those programs are successful in increase adolescents' knowledge about sleep<sup>12</sup> and can produce minor benefits on sleep and mood. However, the

effects are known to be restricted to a short time interval<sup>13</sup>. We tested this approach in young adolescents (13 years old) and we could not find any significant improvement in adolescent's sleep and sleepiness<sup>14</sup>. Alternatively, when parents are setting bedtime, adolescents presented longer sleep duration and reported less fatigue. Likewise, the proportion of adolescents living in homes with parental control of bedtime decays abruptly as they age, making this strategy very difficult to implement<sup>15</sup>. In this context, changing school start time would be the best strategy to tackle adolescent sleep deprivation. The first evaluation of the effect of delayed school start time was performed in the early 2000's<sup>16</sup>. After schools changed their start time from 7:15 AM to 8:40 AM adolescents presented longer sleep duration, less reported depression, and a reduction in school absence was found. However, due the lack of standardized questionnaires and control groups, there is still need for better evaluation of DSST. More recently, other efforts to evaluate the effects of DSST on adolescent's sleep were performed<sup>17-23</sup>. One hour of delay resulted from 45 min increment on sleep duration<sup>17</sup> evaluated by subjective measures, another study with actigraphy found 55 minutes of increase<sup>24</sup> when actigraphy evaluation of sleep was performed. Furthermore, actigraphy based study also found significant increase in sleep duration when classes started 55 minutes later<sup>20</sup>, or between 50 to 60 minutes later<sup>19</sup>. In summary, effects of DSST on sleep duration were reviewed recently<sup>25,26</sup>. The cross-sectional studies reviewed are showing longer sleep duration

when schools are delaying the start time. A very recent study with longitudinal approach found the effects of DSST on sleep duration are sustained during the year<sup>27</sup>.

Evaluating the effects of DSST on emotional health and cognition is crucial to a broader understanding of the outcomes of DSST. Positive associations between sleep duration and emotion during DSST were

confirming our initial hypothesis, we identified a significant delay on wake up time during the interventional week ( $F_{(2,36)}=83.6$ ;  $p<0.001$ ) figure 2B. Participants woke up at  $6:54 \pm 00:12$  during week A, at  $7:42 \pm 00:30$  during week B, and at  $6:46 \pm 00:15$  at week C. Tuckey *post-hoc* test indicated later waking up time during week B in comparison to A and C ( $p<0.001$ ). As a result of the delaying the wake-up time, adolescents



**Fig. 1. Study design.** We used a cross-sectional design with withing-subject A-B-A style with repeated measurements. All measurements were the same for the three weeks. Week A was a baseline with school start times at 7h30 AM. Week B was the experimental one with school starting times at 8h30 AM. In week C, school start time was back at 7h30 AM. On day 1 of week A, students filled up baseline questionnaires on socio-economic status and received instructions about the experiment: how to fill out the KSS, and how to wear their actigraphs. On days five, twelve and nineteen, the Friday of each week, subjects filled out the PSQI, ESS, POMS and HADS questionnaires, followed by two cognitive tasks: Word-list recall and N-Back test>

described<sup>17,28,29</sup>, as well as modest effects on cognition<sup>24</sup>. The above-mentioned studies produced significant evidence in favor of DSST. However, most of them rely on cross-sectional design. A comprehensive evaluation of DSST on cognition considering withing-subject study design is missing. Taking advantage of a within-subject study design (A-B-A design), we evaluated the effect of a one-hour delay on school start time for sleep, sleepiness, cognition, and emotion in high school students. The strength of this study is the presence of two baseline evaluations with an intervention (delayed school start time) in between.

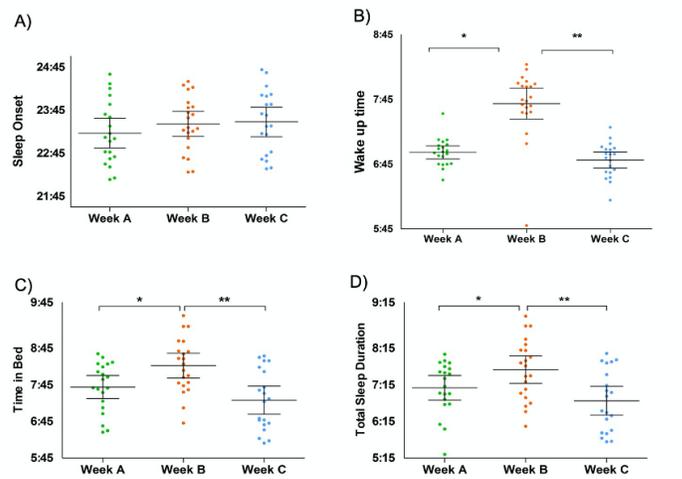
## Results

A total of 48 adolescents (18 female aging on average  $16.0 \pm 0.81$  years old) initiated the study. From those, 17 students were from the first year, 23 of the second and 8 students from the third year of high school. Five students gave up participating during the experiment and another five students were excluded from analysis due to exclusion criteria (four students due to the use of medicine that affects the central nervous system, and one student reported use of cannabis).

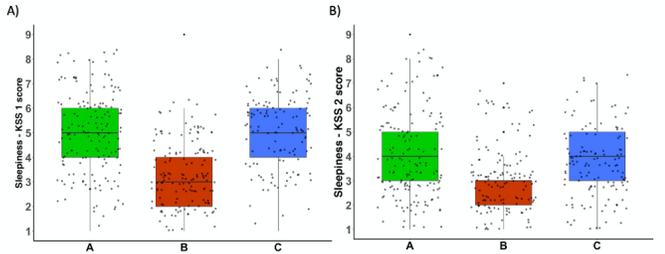
### Sleep-related outcomes

Actigraphy results on bedtime, wake-up time, time on bed and sleep duration are presented in figure 2. We expected to identify a delay in bedtime during the interventional week. However, contrary to our initial hypothesis, there was no significant change on bedtime between the weeks, considering repeated measures ANOVA ( $F_{(2,36)}=1.36$ ;  $p=0.27$ ), Figure 2A. Adolescents went to bed at  $23:18 \pm 00:42$  during week A, at  $23:29 \pm 00:36$  during week B, and at  $23:28 \pm 00:41$  at week C. However,

spend more time in bed during the interventional week ( $F_{(2,34)}=29.2$ ;  $p<0.001$ ; Tuckey *post-hoc* indicate a longer time in bed for week B in comparison to A and C,  $p<0.001$ ). Before the DSST, adolescents spent



**Fig. 3. Sleep timing and duration measured by actigraphy during the schooldays.** Each dot represents a subject, black line and whiskers indicate means and standard deviation, week A is represented in green, week B in orange and week C in blue. There was no significant change in sleep onset time (A) between the weeks. A significant delay in waking up time was identified during week B (B) in comparison to the others. Participants spent longer time in bed during week B (C) and a significant increase in sleep duration at week B (D). Repeated measures ANOVA was performed to compare the effects between weeks. Pairwise comparisons were performed: \* represents comparison between Week A and B and \*\* between Week B and C with significance  $<0.05$ .



**Fig. 3. Subjective sleepiness was evaluated in two moments from Monday to Friday during the three experimental weeks.** As soon as adolescents started their daily activities at school, they performed the Karolinska Sleepiness Scale. A clear reduction on subjective sleepiness is noted during week B in either evaluated moment. Each dot represents a datapoint for a day of the week. Data were collected from Monday to Friday.

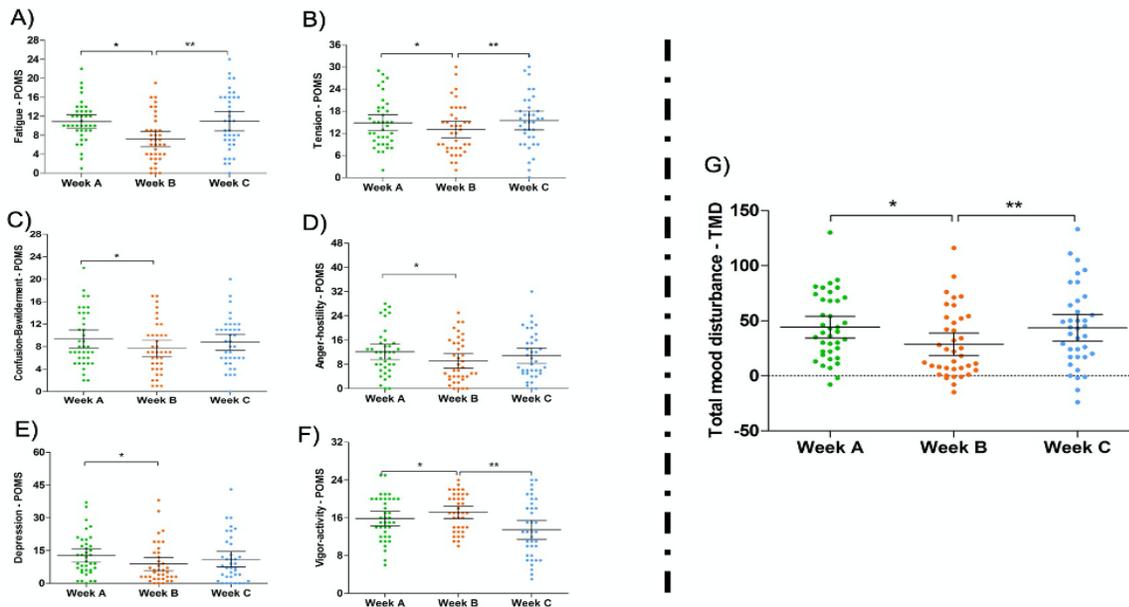
(week A), in mean  $07:33 \pm 0:38$  hours in bed. During the week B, adolescents spent  $08:13 \pm 0:43$  hours in bed, and after school starting time being resumed at 7:30AM adolescents spent  $07:16 \pm 0:47$  hours in bed, figure 2C. Finally, we also tested the sleep duration according to actigraphy. Our results indicate a significant extension on sleep duration at week B ( $07:35 \pm 0:48$ ) in comparison to week A ( $07:03 \pm 0:41$ ) or week C ( $06:46 \pm 0:48$ ) as confirmed by repeated measures ANOVA ( $F(2,34)=22.0$ ;  $p<0.001$ ; Tukey *post-hoc* indicate a longer time in bed for week B in comparison to A and C,  $p<0.001$ ), as displayed in figure 2D.

DSST was effective in reducing the subjective sleepiness evaluated by the Epworth Sleepiness Scale ( $F(2,68)=7.60$ ;  $p=0.001$ ). Subjects reported lower scores for the ESS during week B ( $8.26 \pm 4.15$ ) in comparison to week A ( $10.10 \pm 3.24$ ) or C ( $9.63 \pm 3.62$ ) as confirmed by Tukey *post-hoc* comparison (A-B $p=0.001$ ; B-C $p=0.019$ ).

To further explore the effect of DSST on subjective sleepiness, subjects were asked to fill out a Karolinska Sleepiness Scale (KSS) twice a day during the three experimental weeks. Subjects filled out the KSS as soon as their classes started (KSS-1 at 7h30 for weeks A and C, 8h30 for week B) and just before leaving the school after the final class (KSS-2 at 12:00 for weeks A and C, 12:15 for week B). Results presented at Figure 03 demonstrated the effect of DSST on either moment. Repeated measures ANOVA using the week as between factor and time of day (KSS-1 and KSS-2) as repeated measure yielded a significant effect of the week ( $F(2,456)=58.5$ ;  $p<0.001$ ), time of day ( $F(1,456)=141.8$ ;  $p<0.001$ ) and an interaction between these factors ( $F(2,456)=13.87$ ;  $p<0.001$ ). Adolescents reported being more alert at the beginning of classes during week B in comparison to week A (A-B $p=0.001$ ) and week C (B-C $p=0.001$ ) as depicted in Figure 3. Importantly, subjects presented lower scores for sleepiness when leaving the school during week B, in comparison to week A (A-B $p=0.001$ ) and week C (B-C $p=0.001$ ).

Mood-related outcomes

We found a significant reduction of the scores for the depression component of HADS during week B in comparison only to week A [(HADS-D scores for Week A:  $5.97 \pm 3.01$ ; for Week B:  $4.86 \pm 3.05$ ; and for week C:  $5.49 \pm 3.97$ ); ( $F(2,68)=5.95$ ;  $p=0.004$ ; A-B $p=0.003$ )]. However, we did not find any effect of the DSST on the



**Fig. 4. Profile of mood states outcomes measured during the three experimental weeks according to the subscales.** A) and B) Adolescents reported feeling less fatigue, and tension during week B in comparison to weeks A and C (B-A and Cptukey $<0.001$ ;C); D) and E) A reduction on scores for confusion (C), anger (D), and depression (E) between weeks B and A was found, (B-A)tukey $<0.001$ ). No significant effect between week B and C was found. F) Adolescents reported feeling more vigor during week B in comparison to weeks A and C (B-A and Cptukey $<0.001$ ). G) A significant effect of the DSST was found for the total mood disturbance score where scores for week B are lower than scores for weeks A and C (B-A and Cptukey $<0.001$ ).

### Sleepiness

Excessive daytime sleepiness is one of the most frequent disturbances related to sleep deprivation in adolescents. We found that

anxiety component of HADS [HADS-A scores for Week A:  $7.43(\pm 3.57)$ ; Week B:  $6.46(\pm 3.74)$ ; and Week C:  $7.26(\pm 4.36)$ ; ( $F(2,68)=2.33$ ;  $p=0.105$ )].

During the week of later school start time, adolescents presented significant effects on mood according to the POMS instrument. Figure 4 presents the means for the six components of POMS as well as the total score for the instrument. Adolescents presented less fatigue during the week B in comparison to week A and C ( $F(2,68)=14.86$ ;  $p<0.001$ ; A-B $p<0.001$ ; B-C $p<0.001$ ), Figure 4A. Similar effect was observed for the Tension component ( $F(2,68)=6.89$ ;  $p=0.003$ ; A-B $p=0.007$ ; B-C $p=0.006$ ), figure 4B. In regards of the factor Confusion-Bewilderment a significant effect of the DSST was noted when compared the Week B to Week A only ( $F(2,68)=4.78$ ;  $p=0.011$ ; A-B $p=0.010$ ; B-C $p=0.091$ ), figure 4C. Similar effects was noted for anger-hostility ( $F(2,68)=6.70$ ;  $p=0.002$ ; A-B $p=0.001$ ; B-C $p=0.10$ ), figure 4D, and depression ( $F(2,68)=3.82$ ;  $p=0.027$ ; A-B $p=0.021$ ; B-C $p=0.19$ ), figure 4E. Adolescents also presented higher scores for vigor at week B in comparison to week C ( $F(2,68)=9.64$ ;  $p<0.001$ ; A-B $p=0.21$ ; B-C $p<0.001$ ), figure 4F. In summary, DSST positively changed the total mood disturbance score in adolescents ( $F(2,68)=4.05$ ;  $p=0.022$ ; A-B $p=0.04$ ; B-C $p<0.04$ ) Figure 4G.

### Cognitive-related outcomes

#### Free-Recall task

Subjects did not recall more words during the interventional week considering the performance index [(Week A:  $44.2\% \pm 10.3\%$ ; Week B:  $47.5\% \pm 11.6\%$ ; Week C:  $43.7\% \pm 13.2\%$ );  $F(2,68)=1.62$ ;  $p=0.205$ ].

#### N-Back task

Right after performing the Free-Recall task, subjects started the N-Back task. Table 2 summarize our findings. Our results indicated no effect of the interventional week on total correct responses for the auditory component ( $F(2,66)=2.19$ ;  $p=0.120$ ) neither for the performance index ( $F(2,66)=3.21$ ;  $p=0.047$ ; A-C $p=0.061$ ). However, adolescents presented a better performance for the spatial component during week B in comparison to week A for the number of correct responses ( $F(2,66)=4.12$ ;  $p=0.021$ ; A-B $p=0.019$ ), and marginal effect for the performance index ( $F(2,66)=3.09$ ;  $p=0.052$ ; A-B $p=0.051$ ) with subjects performing slightly better at week B. Results are depict at table 1.

**Table 1.** N-Back performance between experimental weeks for either component, auditory or spatial.

	Auditory		
	Week A	Week B	Week C
Correct Responses	16.0 ( $\pm 2.43$ )	16.8 ( $\pm 2.18$ )	16.8 ( $\pm 3.19$ )
Performance Index	0.71 ( $\pm 0.10$ )	0.76 ( $\pm 0.10$ )	0.76 ( $\pm 0.15$ )
	Spatial		
	Week A	Week B	Week C
Correct Responses	15.6 ( $\pm 2.93$ )	<b>17.1 (<math>\pm 2.66</math>)</b>	16.1 ( $\pm 3.43$ )
Performance Index	0.71 ( $\pm 0.13$ )	<b>0.77 (<math>\pm 0.12</math>)</b>	0.73 ( $\pm 0.162$ )

Note. Repeat measures ANOVA was performed. Significant difference between weeks was found for the spatial component. Adolescents performed better during week B in comparison to week A for the number of correct responses ( $F=4.12$ ;  $p=0.021$ ; Tuckey post hoc  $p<0.05$ ). Marginal significance was reached for performance index ( $F3.09$ ;  $p=0.052$ ) better performance for week B in comparison to week A (Tuckey post hoc  $p<0.05$ ).

**Fig. #.** Profile of mood states outcomes measured during the three experimental weeks according to the subscales. A) and B) Adolescents reported feeling less fatigue, and tension during week B in comparison to weeks A and C B-A and Cptukey $<0.001$ ;C), D) and E) A reduction on scores for confusion (C), anger (D), and depression (E) between weeks B and A was found, (B-A $p_{\text{Tukey}}<0.001$ ). No significant effect between week B and C was found. F) Adolescents reported feeling more vigor during week B in comparison to weeks A and C (B-A and Cp $_{\text{Tukey}}<0.001$ ). G) A significant effect of the DSST was found for the total mood disturbance score where scores for week B are lower than scores for weeks A and C (B-A and Cp $_{\text{Tukey}}<0.001$ ).

### Discussion

In this cross-sectional with within-subject design study, school start time was delayed in one hour (from 7:30AM to 8:30AM). Adolescent's sleep, somnolence, emotional health and cognition were evaluated before DSST, during DSST and after the school resuming its original school start time. Adolescents did not change their bedtime during the DSST; however, they benefit from later waking up time and increased their sleep duration. Complimentary, a significant reduction on sleepiness was found. The outcomes of DSST were beyond the sleep domains, improvements on emotional profile were described as well. Adolescents reported feeling less depressed, angry, tense and fatigue. They reported feeling vigorously and consequently their mood profile was significantly better. Minor improvements on cognition were also noted.

Delaying school start time is not an easy to implement solution. The challenges in addressing and assessing have been reported<sup>37</sup>. Our study overcame one of the biggest challenges, the possibility to evaluate in a longitudinal setting the effect of DSST with two different baseline conditions, before the change and after the remission to regular SST. Our findings are in agreement with previous findings that adolescents would not delay their regular bedtime<sup>19,27</sup>, but delay their waking up time, resulting in longer sleep duration.

As consequence of longer sleep duration adolescents reported feeling less somnolence when arriving and before leaving school. Improvements on sleepiness have also been reported elsewhere<sup>27</sup>. We expected to see effects of the DSST on self-reported sleepiness at the beginning of the school day. Surprisingly, we found that adolescents felt more alert also when leaving school. Our study also found significant improvement on sleepiness measured by ESS scale. The Karolinska Sleepiness Scale is known to be sensitive to sleep restriction<sup>39</sup>. In this way, our interpretation is that adolescents were getting sufficient sleep during the DSST week. Our findings also demonstrate positive changes on emotional health of adolescents. Sleep is an important regulator of emotional health<sup>40</sup> and the association between sleep deprivation and poor emotional health in adolescents has been described<sup>41</sup>. Furthermore, emotion is an important factor modulating school learning<sup>42</sup>. We believe that our findings support the positive effects of sleep on emotional regulation and could reflect in improvements of school attendance and performance. It is possible, however, that an optimistic bias has masked our findings on emotional health. Further longitudinal evaluation would clarify this.

It is well known the association between sleep and memory consolidation<sup>43</sup>. Beyond that, sleep is crucial for renewing learning capacity<sup>44</sup>. Here, we tested memory using a standard paradigm for declarative memory, a free-recall task. We found no significant benefit of DSST on this declarative memory task. A possible explanation is that adolescent's performance improved across weeks and reached a ceiling effect. Working memory also benefits from sleep<sup>41,45</sup>, during the interventional week adolescents performed better on the N-Back task in agreement with previous findings. During weeks A and C, where adolescents presented shorter sleep duration, their performance on N-Back was also reduced in comparison to the week where adolescents presented longer sleep duration.

Beyond the number of positive outcomes of the DSST presented here, we also argue that later school start time is more compatible with chronotype variability. It reduces the pressure on evening-type adolescents on waking up too early and does not suppress the expression of behaviors of early-type. In the current temporal settings,

with schools starting too early, there is a recognizable benefit for early-type subjects to obtain better grades than evening-type 46. This means later school start time also provides a better environment for evening-type adolescents to improve performance.

The findings presented in our study provide additional support to the growing amount of evidence on the positive outcomes of delaying school start time. We postulate that DSST represents a necessary, although not sufficient, countermeasure in order to reduce the negative impacts of sleep restriction in adolescents. We acknowledge the logistical challenges for implementing a significant delay on school start time, but even so, efforts must be made to build a more inclusive educational system.

## Materials and Methods

A high school located in Palotina (Paraná state) Brazil was the setting of the study. The school board agreed with the study design and the protocol. The Federal University of Fronteira Sul ethics board approved the study protocol (CAAE: 88591718.3.0000.5564). Adolescents and their parents signed consent forms previously to the beginning of the experiment. This is a within-subject study (A-B-A study) comprised of three weeks of evaluation. During the first and last week of our protocol, school started at the regular time (7:30 AM). During the second week, the school start time was delayed in one hour, with classes beginning at 8:30 AM. To minimize the school environment's disturbance, the entire school shifted the start time during the intervention week. However, due to the extension of the evaluations, we randomly selected by a draw a subset of students from the three years of high school levels. A total of 48 adolescents were recruited: 17 (7 female) from the first year, 23 (8 female) from the second year and 8 (3 female) from the third year of high school. Participation was voluntary and adolescents could resign from the study at any time. Exclusion criteria included: diagnosed neurological or psychological disease, current use of antidepressant or hypnotic medications.

## Data Collection and Measures

On day one of the experiment, subjects filled out a baseline questionnaire with socio-demographic information and health status and received the actigraph (ActiTrust AT0503, Condor instruments) with instructions on how to use it. Adolescents wore the actigraph on their non-dominant wrist during the three experimental weeks, returning it at the last day of the experiment. Subjective sleepiness was evaluated every school day. Subjects performed the Karolinska Sleepiness Scale<sup>30</sup> when they arrived at school and before left school. Finally, every Friday subjects performed a set of evaluations that included: sleep quality with an adapted version of the Pittsburgh Sleep Quality Index (PSQI)<sup>31</sup> to ask about sleep quality in the past week instead of the past month; subjective sleepiness using the Epworth Sleepiness Scale<sup>32</sup>; emotion with two instruments: the Profile of Mood States (POMS)<sup>33</sup> and the Hospital Anxiety and Depression Scale (HADS)<sup>34</sup>; and cognition using two standard tasks based on the Psychology Experiment Building Language (PEBL)<sup>35</sup>, a Free-Recall task (with 3 lists of 10 words) and the N-Back task (limited to 3-backs and with spatial and auditory information). Figure 1 summarizes our experimental design.

Sleep timing and duration were evaluated by actigraphy during school days. In order to avoid masking effects from Monday and Fridays mean for three days of week (Tuesday, Wednesday, and Thursday) were obtained for: bedtime, wake-up time, time in bed, and total sleep time. Sleep quality was considered as the final score for PSQI. Subjective sleepiness was obtained from the final score of ESS. Evaluation of subjective sleepiness during school days was performed by calculation of the mean for KSS scores according to the moment of evaluation: when subjects arrived at school (KSS1) and when they were about to leave the school (KSS2). The emotional profile was obtained for each component of the POMS scale: Fatigue, Tension, Confusion, Anger-Hostility, Depression, Vigor, and the Total Mood Disturbance score; as well as for both components of the HADS scale: Anxiety and Depression.

Cognition was evaluated by two different tasks using the psychology experiment building language (PEBL)<sup>35</sup>. We used a word-list test with a free-recall procedure. Working memory was evaluated by the N-back test<sup>36</sup>. For either task, subjects were assessed at the end of each week resulting in a 3 times repeated measure. For the Free-recall task subjects were exposed to 3 lists of 10 words each during a learning session. After a short incubation interval, subjects were asked to recall as many words as they remember for each list. We evaluated the performance index (number of words recalled by the number of words presented on each list

in percentage). For the N-Back task we applied a dual-task paradigm using two modalities of stimuli (spatial and auditory). Subjects were familiarized with the test before the first attempt and had one opportunity to perform the task every Friday. We evaluated the total of correct responses for both parameters (spatial and auditory) as well as the performance index (ratio between the number of correct responses and the number of trials).

## Statistical analysis:

We tested within-subject effect of the intervention using repeated measures ANOVA and Tuckey post hoc for pairwise comparisons.

## End Matter

### Author Contributions and Notes

Contributor's statement page

Dr. Beijamini, Dr. Louzada, and Dr Pedrazzoli conceived study design and methodology. Dr. Pedrazzoli was responsible for the actigraphs of the study. Ms Araujo and Ms Bianchin performed data acquisition. Dr. Beijamini, Ms Araújo, and Ms Bianchin performed data analysis and interpretation. Dr. Beijamini was responsible for manuscript preparation. All authors revised the final version of this manuscript.

The authors declare no conflict of interest.

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