Sleepless in Seattle: How Human Sleep Patterns Kept Me Up At Night

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Abstract

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There is a sleep deprivation epidemic in the United States, particularly amongst teens and young adults. During puberty, teens undergo biological changes to their circadian system that leads to a preference for later bedtimes and wake times. However, societal pressures for high school and college courses force these groups to wake early in the morning for classes. Caught between the biological drive for later bedtimes and social drive for early rise times, teens and young adults reduce their nightly sleep to unhealthy levels. Over time, sleep deprivation can have devastating effects ranging from unstable mood to impaired learning and memory. For my thesis, I examined the ways that sleep patterns affected lifestyle outcomes including academic performance, mood, daytime sleepiness, and other variables in two field studies of local populations: high school students from the Seattle School District and undergraduate students from the University of Washington.

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The world beats to a natural rhythm. The sun rises and falls, tides come in and out, the moon waxes and wanes, and for much of the world seasons change gradually. For almost all life on this planet, these rhythms come with significant challenges. With the sunrise come changes in temperature and light, with the rising of the tides come changes in water level, temperature and salinity, and with the changing of the seasons can come swings in temperatures, rain fall, daylight hours, and much more. To increase fitness in response to these cycles, nearly every living being has evolved a mechanism to anticipate these changes. These 'biological clocks' sustain rhythms of physiology and behavior and have three main properties: they exist within the organism (they are endogenous), they can synchronize their time-keeping with the environment (they can be 'entrained'), and they maintain their integrity regardless of the external temperature (they are temperature compensated)[1]. From cyanobacteria to humans, these clocks have evolved as one of the most important conserved traits of life on earth.

The Circadian Clock

For virtually all organisms the most important timing system is the circadian clock, which anticipates the day/night cycle. In the most basic form, a biological circadian timing system has three main components (Figure 1)[2]. At the core is the clock itself, which generates rhythmicity through a transcription-translation feedback loop (TTFL), a system whereby expression of so-called clock genes creates proteins which negatively feedback on their own promoters. As those proteins break down, their genes can be promoted again, and the cycle repeats. While the clock has the ability to generate a self-sustaining rhythm in the absence of environmental cycles its period is not exactly 24 h. Thus, in organisms living in their natural environment the clock receives cyclic environmental signals through sensory systems that allow the clock to modulate its timing to match that of the environment, a process called entrainment. The clock then sends signals to the rest of the organism to modulate processes like hormone release, gene expression, and behavior.

The master circadian clock in humans is located in a bundle of ~80,000 cells called the suprachiasmatic nucleus (SCN) within a region of the brain called the hypothalamus. Each of these cells expresses two clock genes, *Clock* and *Bmal1*, and their products CLOCK and BMAL1

dimerize to act as promoters for a collection genes responsible for core clock function as well as for other genes—clock-controlled genes—that regulate rhythmic cellular functions (Figure 2)[2]. Among the clock genes regulated by CLOCK and BMAL1 are the genes of the *Per* and *Cry* families. CLOCK-BMAL1 activation of *Per* and *Cry* genes leads the to the synthesis of PER and CRY proteins, which will in turn inhibit the ability of the CLOCK-BMAL1 dimer to stimulate *Per* and *Cry* transcription. The cyclic degradation of PER and CRY proteins will restart the 24 h cycle of transcription activation and inhibition.

Mutations in these and other core clock genes can lead to observable phenotypes in humans. For example, mutations in the *Per* genes are associated with Familial Advanced Sleep Phase Syndrome(FASPS)[3,4]. This variant causes individuals with the mutation to have greatly advanced chronotypes, a term used to describe the diurnal preferences of an individual[5]. Those with FASPS have rhythms that are advanced up to 4 hours, making them extreme early chronotypes, or morning larks. Other variations of *Per* are associated with advanced and delayed sleep phase syndromes (ASPS/DSPS)[6]. Furthermore, it is not just extreme phenotypes that have been linked to clock gene variations. Smaller differences in chronotypes, differences of 45 minutes instead of 4 hours, have been linked to polymorphisms in the *Clock* gene[7].

The TTFL is sufficient to generate a rhythm in isolation from any external cycles; however, most organisms do not live in constant conditions and thus must entrain the internal clock to sync with the environment around it. For most species, including humans, the natural light-dark (LD) cycle is the most prominent entraining cycle. Light has the ability to change the phase of the human circadian clock, advancing it in the morning and delaying it in the evening[8]. While natural light is the strongest entraining signal for humans, studies have shown that even low levels of indoor light are sufficient to reset the phase of the human circadian clock[9,10]. Humans typically have an endogenous period that is greater than 24 hours[11]. In order to remain entrained to environment the human clock must advance its phase daily, making morning light critical to the human circadian system and evening light potentially disruptive (see below).

The mammalian retina has multiple light-sensing cells within their retinas to convey information to the brain[12,13]. In addition to the commonly recognized rod and cone photoreceptors, a subset of retinal ganglion cells (RGCs) in vertebrates are also intrinsically

photoreceptive (ipRGC). RGCs were previously thought to be middle-men in the visual pathway, receiving information from rods and cones and sending it to retino-recipient areas in the brain. However, it is now known that ipRGCs, which may represent 2-4% of all RGCs, contain their own photoreceptive pigment called melanopsin and are capable of processing light information in the absence of rod and cone input. ipPRGs integrate rod and cone input as well as their intrinsic response to light and send this information to centers of the brain that are typically not involved in image-forming photoreception. One of these centers is the SCN, which receives dense projections from ipRGCs[12,13,14]. Because of the prevalent role of the LD cycle in the entrainment of circadian rhythms this system has evolved to be so highly sensitive to light , and even relatively low-intensity artificial light has the ability to entrain the human circadian system[15,16,17,18], a phenomenon that has serious implications for human health[19,20,21].

Sleep Timing and Sleep Stages

One of the most important circadian outputs in animals is the control of sleep, a natural and reversible state of unconsciousness that is accompanied by a reduced response to stimuli and general inactivity of the body. Sleep in mammals is broken up into two main stages: slow-wave sleep (SWS), characterized by low-frequency (~1 Hz) oscillating high-amplitude EEG waves during non-rapid eye movement (non-REM) sleep, and REM sleep, characterized by fast oscillating low-amplitude EEG waves and muscle atonia that involves most muscles excluding respiratory muscles and oculomotor muscles that produce rhythmic eye movements, hence its name. In addition, human beings exhibit a third type of non-REM sleep ("N2") characterized by sleep spindles and other brain waves that are distinct from both REM sleep and SWS[22]. During a typical nocturnal sleep bout, these stages cycle with a period of approximately 2 h, with the early portion of the night dominated by SWS-rich cycles and the late portion dominated by REM-rich sleep cycles (Figure 3).

In addition to differences in EEG waves, SWS and REM are also controlled by two different driving forces. Originally proposed in 1982 by Alexander Borbély, the Two-Process Model of Sleep hypothesized that SWS and REM were largely controlled by a homeostatic and circadian process, respectively (Figure 4)[23]. The homeostatic drive for sleep (Process S) is a

measure for how long a person has been awake. Upon waking, sleep pressure begins to build and will continue to do so until an individual relieves that pressure by sleeping. If you sleep deprive an individual their sleep pressure will continue to increase. During a recovery sleep bout, previously sleep-deprived individuals show increased SWS that is correlated with the time spent awake[24]. Additionally, in both human and animal models it has been shown that areas of the brain with high activity during wake increases the amount of SWS recorded from that area during subsequent sleep bouts. For instance, humans that spend a day using only one arm while the other is immobilized show increased SWS in the brain areas associated with the active arm and decreased SWS in the areas associated with the inactive arm[25]. Similar data have been collected from various animal and human studies which preferentially target motor, visual, and other areas of the brain[22,26].

The circadian drive for wakefulness is an output of the clock and is controlled by signals from the SCN. Unlike the homeostatic drive for sleep the circadian drive is cyclical across a ~24-hour period. Importantly, although the process C is symbolized as a sinusoidal oscillation in a wake-promoting process, the circadian system promotes both wakefulness during the daytime and sleep during the night; furthermore, the peak of wakefulness does not occur precisely at the middle of the day (noon), but instead in the evening. As Figure 4 shows, the distance between Processes C and S changes over time. During the early night, when the homeostatic drive for sleep is high and the circadian drive for wakefulness is falling, the body reaches a "sleep gate" and sleep is initiated (11 pm in Figure 4). As the homeostatic drive for sleep falls during the night, the circadian drive for wakefulness reaches a nadir and keeps the individual asleep until the two lines meet in the early morning, when the individual wakes up. In the same way that SWS is favored ty increased homeostatic sleep pressure REM sleep is favored an increased circadian sleep drive (the trough of process C in Figure 4). In healthy individuals entrained to a regular light LD cycle, these two processes complement one another to help them fall asleep at the beginning of the night and stay asleep over the course of the night. Furthermore, the out-of-phase peaks determine that earlier sleep cycles be rich in SWS (high homeostatic drive) and late cycles be rich in REM sleep (high circadian drive to sleep).

Sleep and Learning

Nearly all animals studied to date show the presence of sleep or sleeplike states, a remarkable behavior given the relative disadvantage of entering a state of decreased responsiveness to potentially adverse stimuli[27]. While the exact function of sleep is still being debated, it is clear that sleep plays an essential role in the fitness of an organism as virtually every biological function is impaired by sleep deprivation. Loong-term partially sleep deprived animals show progressive decays in health and ultimately die from infections and tissue lesions[28]. Total sleep deprivation in rats led to a 100% mortality rate, although the anatomical cause of death was unable to be precisely determined[29]. Finally, sleep deprivation in humans causes several mental, emotional, and cognitive problems[30], although a total sleep deprivation study has not been completed for obvious ethical reasons.

The positive effects of sleep have been documented for biological mechanisms ranging from immune function[31] to cellular repair[32]; however, one of the best characterized functions of sleep is its role in memory. The ability to form memories requires three main processes: the ability to acquire new information, to consolidate that information for short- and/or long-term storage, and then to retrieve that information as needed. While acquisition and retrieval may be indirectly influenced by sleep insofar as a well-rested brain is a more alert brain, the focus of the majority of research over the past century has been on the ways the sleeping brain consolidates acquired information for medium and long-term storage[22,33,34]. Memories can only be consolidated at the same time that the brain is not acquiring information, thus sleep appears to represent an ideal “off-line” state for memory consolidation. For example, in one study subjects were asked to memorize a list of word pairings then broken into two groups: one that was allowed to sleep at a normal night time after the learning session, and one that was sleep deprived overnight and allowed a recovery sleep period the following morning[35]. Even though the groups got the same total amount of sleep, the sleep deprived group performed significantly worse than the normal group on the declarative task, illustrating the positive effect of sleep after learning for the rapid consolidation of memory.

One of the corollaries to emerge from the vast body of work on sleep and memory is the dual process hypothesis that postulates that different sleep stages serve to consolidate

different kinds of memory. Consolidation of declarative memories, which includes episodic (events in context of time) and semantic (facts) memories, is usually associated with SWS and other EEG signatures of non-REM sleep. For example, in one of the earliest studies on the subject individuals who slept early in the night and got SWS-rich sleep performed better on a declarative word-pair task than individuals who slept late at night and got more REM-rich sleep[36]. In contrast, nondeclarative memories, which include procedural, motor, and perceptual skills, are usually associated during REM sleep. For example, recalled of mirror-tracing skills improved in individuals with late-night sleep (dominant in REM) compared to early night sleep[37]. While the dual hypothesis provides a compelling way to interpret the importance of sleep stages, there is also evidence that the sleep stages work sequentially. In this case, SWS serves as the first stage where memories are re-activated and redistributed throughout the brain, followed by REM sleep where memories are consolidated in the cortex of the brain[38,39]. Ultimately, regardless of the roles of the individual stages of sleep, the healthy timing and organization of sleep is critical for normal memory consolidation.

Beyond the role of sleep in memory, sleep deprivation has obvious detrimental effects on performance. In a classic study by Van Dongen and collaborators, individuals were partially sleep deprived for two weeks and then tested each day on a procedural task (an objective measurement of performance) and asked to rate their perceived performance (a subjective measure of performance)[40]. Over the course of two weeks, participants objective performance steadily declined until the end of the study. Interestingly, after several days the same individuals reported their subjective performance to be stabilizing, indicating that humans may be inherently poor judges of their own performance.

Artificial Light, the Circadian System and Sleep

The advent of electric lighting revolutionized the way human beings live and work; however, it has come at a cost. Over the past century access to cheap electricity has become universal in the developed world[41] while at the same time there is evidence that the sleep duration in adults is declining[42]. This is largely due to access to electrical lighting, which negatively affects human circadian rhythms and sleep in three major ways. First, at an acute

level, light is a naturally arousing stimulus and it allows humans to extend activities into the night when they would previously be preparing for sleep[43]. Second, as indicated above, light in the early night delays the human circadian clock, which will cause circadian rhythms such as the timing of sleep to delay relative to the natural LD cycle. Finally, light directly inhibits the release of the hormone melatonin[44]. Melatonin is released by the pineal gland in response to signals from the SCN as a night hormone, and the onset of its release is one of the main signals for the body that the time for sleep is approaching. Under natural conditions, melatonin release typically starts several hours before bedtime. Artificial light from lightbulbs, televisions, phones, and other sources can inhibit this release and leave the body unprepared for sleep when an individual finally decides to go to bed. Furthermore, beyond the acute inhibition of melatonin release, artificial evening light will also delay the circadian rhythm of melatonin release, shifting the natural marker of the beginning of the night to a later time[16,17,18].

Evidence for a relationship between electrical lighting and human sleep and circadian rhythms has grown over the past decade. Research on two geographically and genetically close groups of native Argentinians has shown that the group living with access to electricity sleeps less each night and has a delayed circadian rhythm relative to the group that lives without electricity[45]. In the United States, researchers monitored sleep and circadian rhythms in a group of students during one week of normal living (electric light condition) and one week of camping (non-electric light condition)[46]. The results were similar to that of the Argentinians: access to electric lighting delayed circadian rhythms and shortened sleep. Interestingly, in a follow-up study the same researchers showed that a single weekend of camping was sufficient to realign the circadian rhythms of individuals to the natural environment[47]. Results from these and other studies have helped elucidate the unintended impact that cheap, accessible electricity is having on human sleep in the modern and modernizing world[48,49] .

As the understanding of the interaction between artificial lighting and circadian rhythms has grown, so has the exposure of other factors that disrupt our circadian rhythms and sleep directly. For instance, exposure to light during the natural nighttime is typically associated with odd work schedules. Medical residents working extended (at least 24 hours of continuous work) shifts more frequently make significantly more errors, including errors that lead to death

in patients, than when these shifts are less frequent[50]. The effects of extended or unusual work schedules go beyond decreased job performance; individuals who work night shifts long-term have higher incidents of mental health disorders, diabetes, and heart disease than those working day shifts, and part of these effects could be mediated by the lack of exposure to natural daylight and the exposure to light during the night[51–53].

School Schedules, the Circadian System and Sleep

The negative impact of schedules that are at odds with our circadian system are not limited shift work. If unusual work schedules lead to poor performance and negative health outcomes, one would expect that school schedules that are not aligned with the circadian biology of students may have similar consequences. Research on the impact of sleep schedules and student success began in the late 1990's when Dr. Mary Carskadon and colleagues, interested in adolescent health, began investigating the ways in which teens were sleeping over time[54,55]. In 1998, Drs. Carskadon and Wolfson published one of the first studies using self-report data that showed that teens in Rhode Island were sleep-deprived[56]. Furthermore, they found that students not getting adequate amounts of sleep reported lower grades, increased daytime sleepiness, and increased prevalence of mood disorders. While this study was limited by the use of self-report data, it did shine a light on the impact that inadequate sleep may have on students living in real-world situations. It also raised concerns about how a preference for early morning school times could exacerbate these problems. Importantly, these and later studies established that during puberty teens undergo several developmental changes, one of which is a shift to have late chronotypes[57]. This is due to changes in both processes that control sleep timing. First, teens typically have a longer circadian period, which delays the circadian drive for sleep (process C). Second, sleep pressure from the homeostatic drive (process S) accumulates more slowly. Finally, teens have a reduced response to early morning light which reduces their ability to advance their clocks. Together, these three effects lead to a naturally delayed sleep timing and preference for later activity.

In the years since, it has become clear that students across education levels are chronically sleep deprived[58], largely due to a mismatch between societally imposed school

schedules and biology. Modern society has been designed to benefit those with an early chronotype; most schools begin at times that are too early and this is incompatible with the adolescent chronotype. This mismatch between the biology and society forces teens between a rock and a hard place resulting in reduced sleep[59]. In 2014, the American Academy of Pediatrics formally named teen sleep deprivation in the United States to be an epidemic and recommended schools for adolescents should start no earlier than 8:30 AM[60].

Scope of this Thesis

In this thesis I will report on two studies which attempt to get at the heart of this issue: how students are sleeping and how sleep affects their daily lives. In the first chapter I will report on the results of delaying secondary school start times for Seattle Public Schools to better align school start times (SSTs) with teen biology. In the second chapter I will report on a multi-year study examining the way that undergraduate students at the University of Washington sleep, what are reliable predictors of their daily sleep pattern and in turn how are specific sleep patterns associated with their academic success. Together, results presented in this dissertation supports that societally imposed school schedules directly impact student sleep and that in turn specific daily sleep patterns are associated with academic performance.

Figures

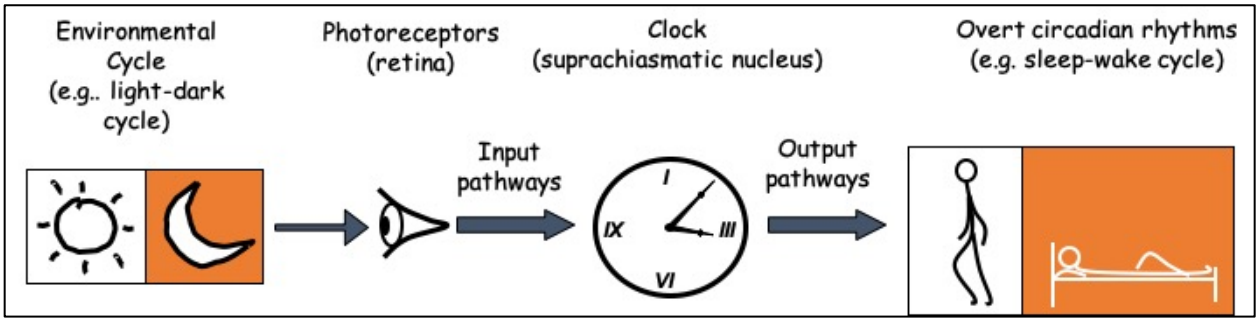

Figure 1 Main components of the circadian clock in humans. A circadian oscillator with a period close to 24 h works as a clock and sustains rhythms of physiology and behavior. Input from environmental cycles, predominantly the LD cycle, entrains the clock to the solar day.

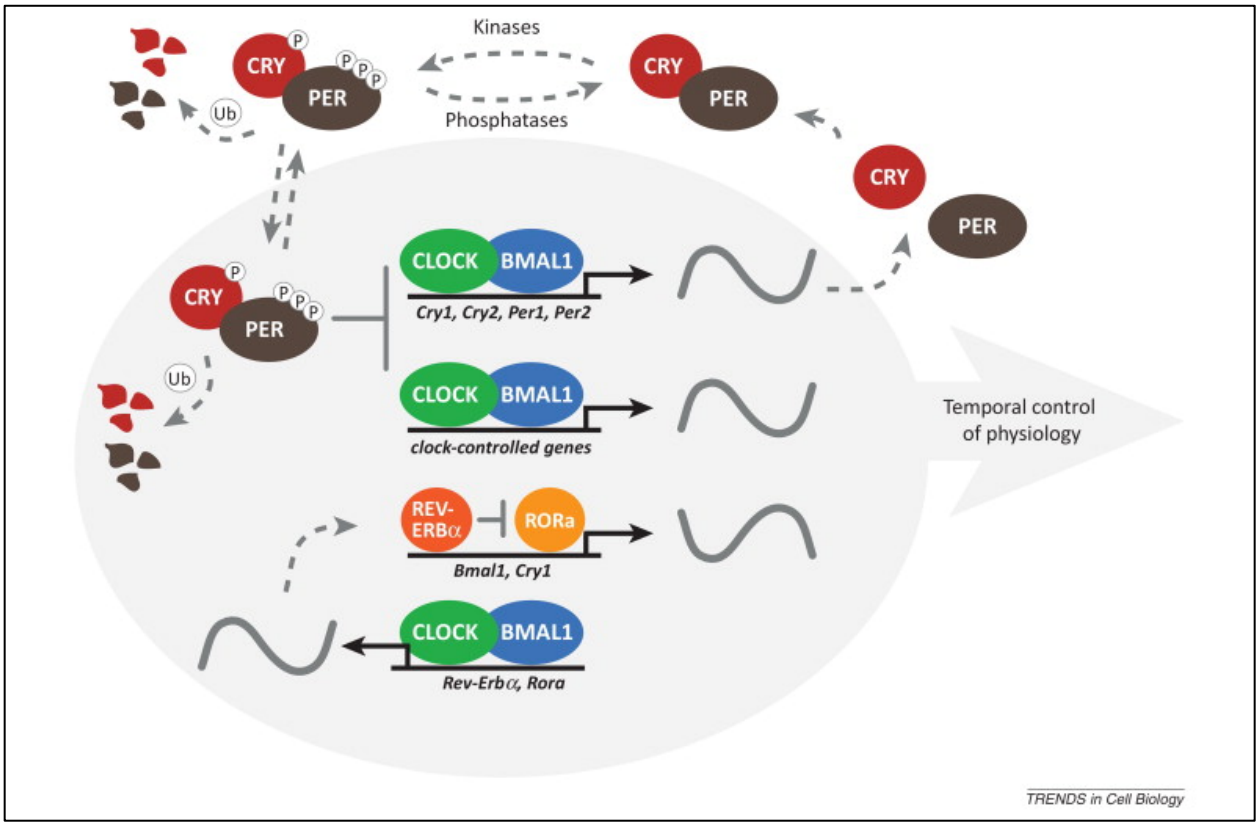

Figure 2 Transcription-Translation Feedback Loop (TTFL) in mammals[2]. The products of the clock genes *Clock* and *Bmal1* dimerize and act as transcription factors to promote the expression the clock genes of the *Cry* (*Cry1* and *Cry2*) and Per (*Per1* and *Per2*) families, as well as clock-controlled genes. PER and CRY proteins dimerize and inhibit the CLOCK-BMAL1 action, thus inhibiting their own transcription. Degradation of PER and CRY proteins after posttranscriptional modifications restarts the ~24-h cycle.

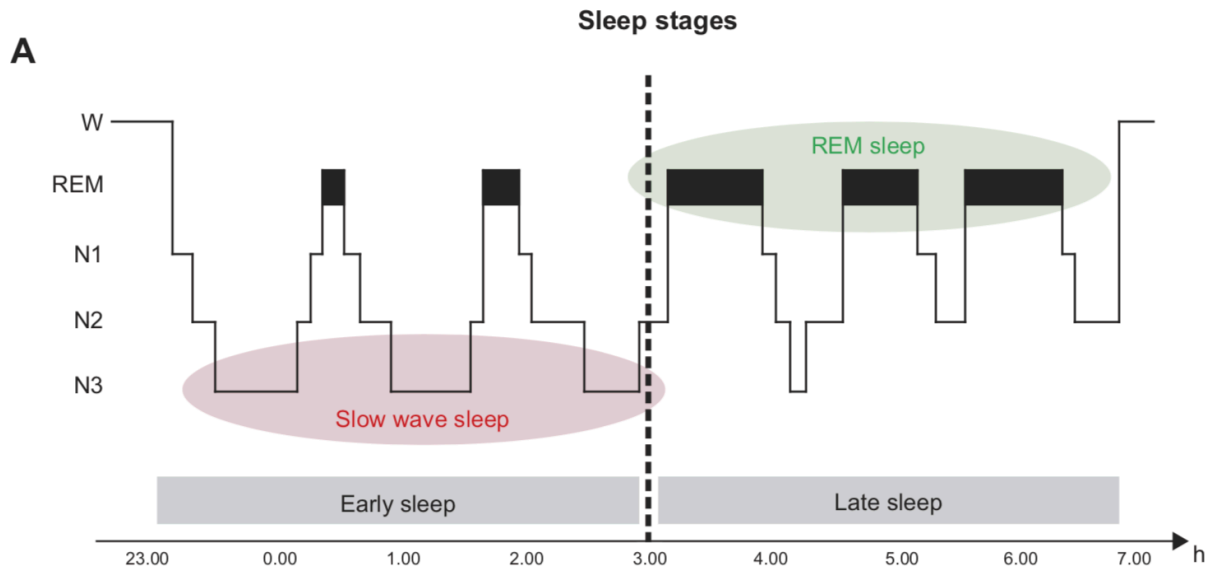

Figure 3 Oscillations between SWS and REM during a typical nocturnal sleep bout[22]. Sleep is manifested in ~2-h cycles that oscillate between non-REM sleep (N1, N2 and N2 stages) and REM sleep; SWS is predominant within N3. Because of the respective regulation by the S and C processes (see text) the earlier sleep cycles of the nocturnal bout of sleep are richer in non-REM sleep and SWS while the later cycles are richer in REM sleep.

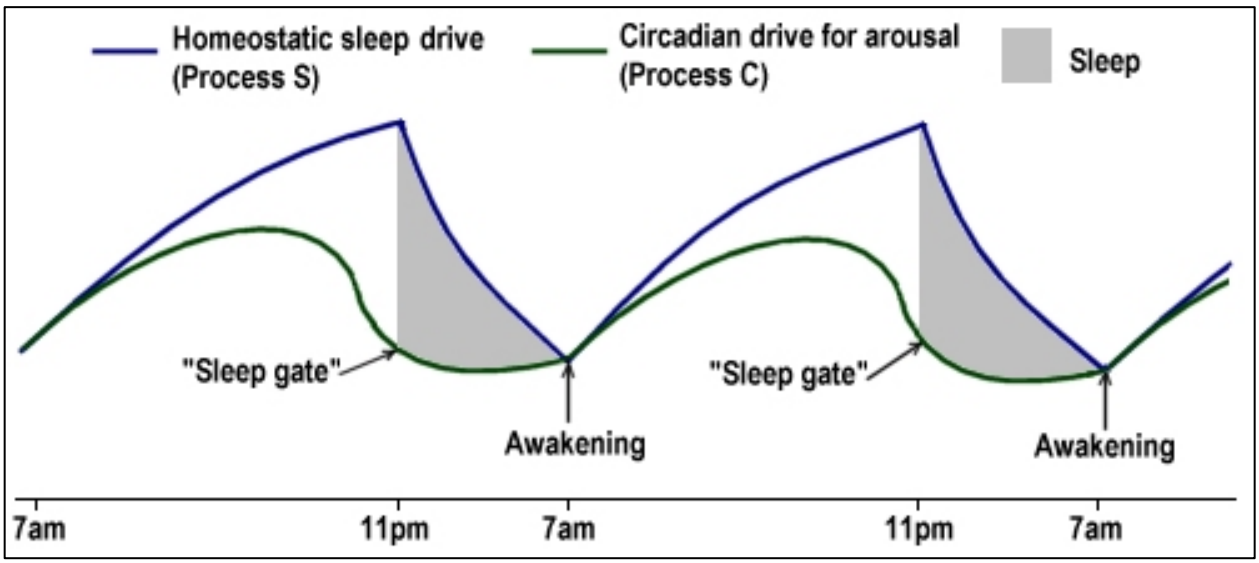

Figure 4 The Two-Process Model of Sleep Regulation. Sleep timing is the result of the interaction between a homeostatic pressure to sleep that increases with wakefulness (Process S) and an oscillation in wakefulness promotion that is driven by the circadian clock (Process C). For details see main text.

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DEVELOPMENTAL NEUROSCIENCE

Sleep*more* in Seattle: Later school start times are associated with more sleep and better performance in high school students

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Most teenagers are chronically sleep deprived. One strategy proposed to lengthen adolescent sleep is to delay secondary school start times. This would allow students to wake up later without shifting their bedtime, which is biologically determined by the circadian clock, resulting in a net increase in sleep. So far, there is no objective quantitative data showing that a single intervention such as delaying the school start time significantly increases daily sleep. The Seattle School District delayed the secondary school start time by nearly an hour. We carried out a pre-/post-research study and show that there was an increase in the daily median sleep duration of 34 min, associated with a 4.5% increase in the median grades of the students and an improvement in attendance.

INTRODUCTION

Adolescents typically have a preference to stay active until late in the evening and to wake up late in the morning. This timing of daily activity or "chronotype" is not only a consequence of a change in social life and the use of electronic devices that keep teenagers awake during the evening, but is also a result of changes in both the circadian and homeostatic regulation of sleep (*1*). During puberty, the adolescent circadian system naturally delays the onset of sleep to a later time. One reason for this is an apparent lengthening of the circadian period during the teenage years (*1*), which typically leads to a later onset of the biological night relative to the light-dark cycle (*2*). Furthermore, there is evidence that the adolescent circadian clock is less sensitive to light during the morning when light advances the circadian clock and its timing of sleep (*1*). On the other hand, the homeostatic regulation of sleep, which increases sleep pressure with waking hours, is also modified in older adolescents. This allows them to stay awake longer, relative to younger adolescents, due to a decreased sleep pressure during wake periods (*3*, *4*).

On the basis of these measurable changes in sleep regulation, adolescents find themselves caught between two competing yet equally important forces: their circadian and homeostatic regulation of sleep, which delays sleep onsets, and their social obligations, which impose early sleep offsets resulting in a net decrease in daily sleep. Most adolescents sleep less than the recommended daily sleep at this age (8 to 10 hours) (*5*, *6*), and an intervention that has been proposed to increase sleep is delaying school start times (American Academy of Pediatrics, 2014). Although some studies have used survey data to show that when teens are allowed to go to school later, they report longer sleep times, so far, there is no objectively recorded data indicating that delaying the school start time lengthens daily sleep in adolescent students.

Increasing daily sleep duration in adolescents is not only critical because of the clear adverse physical and mental health outcomes associated with chronic sleep deprivation but also because of the role that normal sleep plays in learning and memory consolidation (*7*). Any action that results in longer daily sleep duration should also result in better academic performance. The link between longer sleep and better school performance has been hard to establish in field studies; whether delayed secondary school start times result in better performance also remains to be determined.

RESULTS

The ideal field experiment to study the potential benefits of later school start time on both sleep and academic performance should include schools that switched from an early start to a late start (or vice versa), in which students of the same grade, taking the same classes, could be studied objectively. The Seattle (WA) School District decided to delay the start time for secondary schools from 07:50 to 08:45 a.m. This change was implemented for the 2016–2017 academic year and allowed us to conduct a pre-/post-study in which we measured sleep-wake cycles using wrist activity devices (Actiwatch Spectrum Plus, Philips Respironics) during the spring of 2016 (pre) and the spring of 2017 (post). The study populations included sophomores of two public high schools in Seattle. In each year, at the same time of the year, an independent sample of students taking the same science class was studied in each school. The study was implemented as a science laboratory practice in which the students could test predictions about their own sleep patterns. Both the Human Subject Division at the University of Washington and the Seattle Public School District Board approved our study. As part of the 2-week recording phase, each student wore an Actiwatch and completed a sleep diary (used to validate the Actiwatch data), the Beck Depression Index II (BDI-II) Questionnaire (*8*), the Epworth Sleepiness Scale Questionnaire, and the Munich (*9*) and Horne-Östberg (*10*) Chronotype Questionnaires.

Figure 1A presents the wrist activity mean waveforms for students pooled from both schools during each year. During school days, a two-way analysis of variance (ANOVA) yielded an effect of time [$F_{(143,\ 25,311)} = 224.8$, $P < 0.0001$], no effect of year, and an effect of the interaction [$F_{(143,\ 25,311)} = 18.43$, $P < 0.0001$]. Similar effects were found for the nonschool days [$F_{(143,\ 25,025)} = 161.5$, $P < 0.0001$ for time; and $F_{(143,\ 25,025)} = 2.19$, $P < 0.0001$ for the interaction]. However, multiple comparisons revealed that most of the differences in activity between the 2 years emerged from a different wakeup time during

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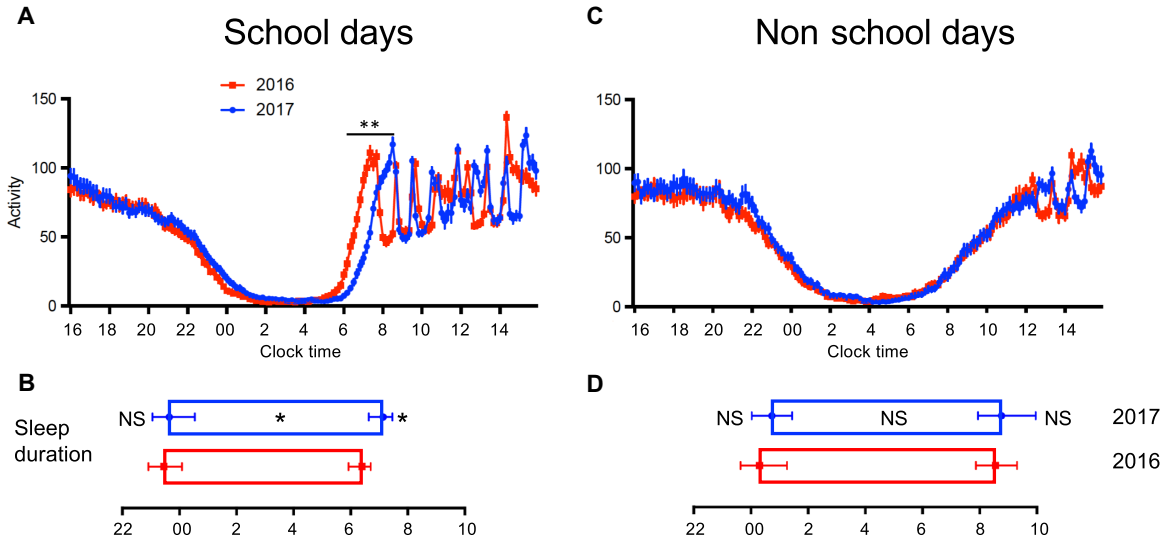

Fig. 1. Delayed school start times result in later sleep offset and longer sleep. Mean student activity waveforms and sleep summaries between years for school (**A** and **B**) and nonschool days (**C** and **D**). For both (A) and (C), there was a significant effect of time, year, and the interaction (see text). $^{**}P < 0.01$, difference between years (Sidak's comparisons). For (B), there is a significant delay in sleep offset ($P = 0.0007$), but not sleep onset ($P = 0.0459$), on weekdays in 2017 as compared to 2016, resulting in a significant increase of sleep duration on school days in 2017 ($P = 0.0007$); $P < 0.017$ threshold for significance for Wilcoxon signed-rank test corrected for multiple comparisons. The same analysis of sleep parameters on nonschool days shows no difference between years (D) [$n = 84$ (2017, school day) and $n = 94$ (2016, school day); $n = 76$ (2017, nonschool day) and $n = 81$ (2016, nonschool day)]. For (B) and (D), values represent median, and bars represent interquartile range. Sleep offset was also tested through generalized linear models (see text). Each student contributed at least five nights for the school-day data and three nights for the nonschool data. NS, not significant.

school days but not during nonschool days. That is, there was a clear difference in the timing of activity between years during school days but not during nonschool days. Notably, the regular peaks observed during the waking hours of school days reveal the break times between classes when students walk from one classroom to another. Analysis of the estimated sleep onset, offset, and sleep duration (see Materials and Methods) confirmed this interpretation. Wilcoxon signed-rank tests corrected for multiple comparisons revealed that students in 2017 had a 44 min later median sleep offset ($P < 0.0001$, effect size = 0.194), and a trend toward a later sleep onset that was not statistically significant. This asymmetric effect led to an overall 34-min increase in the sleep duration median during school days in 2017 ($P = 0.0007$, effect size = 0.353). In contrast to school days, nonschool days did not show any significant differences between years in any of these sleep parameters. The number of naps (counted after inspecting every actogram) students took was very similar between 2016 (152 total naps, 0.6 naps per student) and 2017 (150 total naps, 0.56 naps per student).

We also examined the change in social jet lag, a measure of the difference in sleep timing on school versus nonschool days. We predicted that given the delayed sleep pattern of students on school days in 2017, their sleep during school days would more closely align with their sleep on nonschool days. After controlling for oversleep during nonschool days, because of the accumulated sleep debt during the school days (see Materials and Methods), we observed a significant decrease in social jet lag in students from 2017 (median = 1.25, $n = 76$) as compared to 2016 (median = 1.60, $n = 81$; Wilcoxon signed-rank test, $P = 0.0118$, effect size = 0.616). Social jet lag was also evident when the sleep onset of students was compared between the night from Sunday to Monday and the night from Monday to Tuesday. On both years, Wilcoxon matched-pairs, signed-rank tests revealed that, compared to Monday nights, sleep onset was later ($P = 0.0021$ for 2016 and $P = 0.0003$ for 2017) and sleep duration was shorter ($P = 0.0234$ for 2016 and $P = 0.0142$ for 2017) on Sunday nights; no differences were found for sleep offset. The reduction in social jet lag after the school start time delay further emphasizes the conclusion that later school start times allow students to better align sleep on school days with the circadian timing of their sleep.

A potential outcome of delaying school start times is that a trend for students to go to bed later could lead to exposure to artificial light later in the evening, which could in turn delay the master circadian clock. Figure 2 (A and B) shows the waveforms for exposure to light during different years. Visual inspection of the profiles during school days suggests that, compared to 2016, students in 2017 started their exposure to brighter light intensities later in the morning but did not necessarily end their exposure to bright light later in the evening. Furthermore, in both years, students appear to have a delayed exposure to light on nonschool days compared to school days. Because light intensities students are exposed to vary greatly—indoor light is several orders of magnitude lower intensity than outdoor light—light exposure never showed a normal distribution even after data transformation, precluding us from running a two-way ANOVA. Furthermore, Actiwatch light measurements are typically inaccurate at lower light intensities [see the Supplementary Materials and (*11*, *12*)]. A more meaningful measure of the time course of light exposure is to assess when a student was exposed to a specific light threshold for the first time and the last time each day. We chose a 50-lux threshold as it is just above the threshold for inhibition of melatonin release (*13*). A two-way ANOVA with factors year and day of week (school versus nonschool) of time of first or time of last exposure to 50 lux yielded an effect of year and of day of week but not of interaction (Table 1 and Fig. 2C). Sidak's comparisons

Table 1. Two-way ANOVA results for first and last time of daily exposure to 50-lux light intensity.

	Year		Day of the week		Interaction	
First daily 50-lux light exposure	$F_{(1, 331)} = 18.2$	$P < 0.0001$	$F_{(1, 331)} = 258.1$	$P < 0.0001$	$F_{(1, 331)} = 3.7$	$P = 0.0557$
Last daily 50-lux light exposure	$F_{(1, 331)} = 6.2$	$P = 0.0136$	$F_{(1, 331)} = 111.0$	$P < 0.0001$	$F_{(1, 331)} = 0.01$	$P = 0.92$

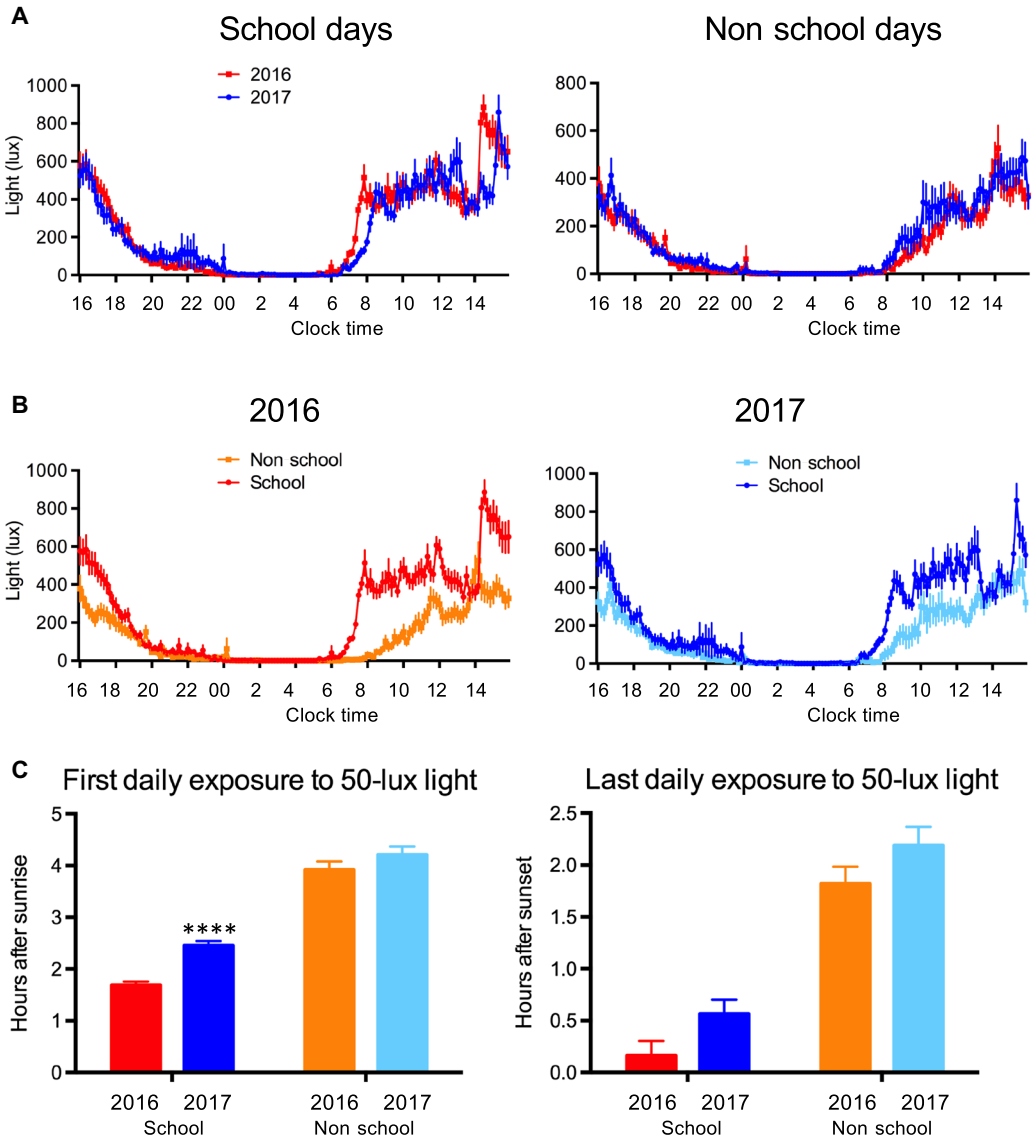

Fig. 2. Delayed school start times result in later exposure to light in the morning but not in the evening. (**A**) Mean student light exposure waveforms between years for school and nonschool days. During school days, students appear to have a delay in morning light exposure but not in evening light exposure. This delay is not evident in the data from nonschool days. (**B**) For both years, exposure to light is delayed in weekends relative to weekdays. (**C**) Because of the non-normal nature of the light data, the times for first and last exposure to 50-lux light on school, and nonschool days were tested for each year using a two-way ANOVA. There was a significant effect of day of week (school or nonschool) and year but not of the interaction (see Table 1); ****$P < 0.0001$, significant difference between years (Sidak's multiple comparisons). No difference was observed on nonschool days nor in the timing of the last daily exposure for school or nonschool days.

for school days demonstrated that while the first daily exposure to 50-lux light intensity occurred later in 2017 than in 2016, there was no difference between years in the last daily exposure to the same light intensity. In both years, comparisons revealed that students' first and last daily exposure to 50-lux light intensity occurred later during nonschool days than during school days (Fig. 2C).

We used generalized linear models with years as the independent predictive variables to determine which dependent variables were significantly different between years. Because of the interdependence among sleep parameters (onset, offset, and duration), we tested one of these sleep variables separately in each model with the remaining parameters (school, sleepiness, depression index, chronotype, and grades). The final, best-fitting model included school, school day sleep offset, academic performance, mood, chronotype, and sleepiness, with sleep offset ($P = 2.8 \times 10^{-5}$; median_{2016} = 06:24 a.m., median_{2017} = 07:08 a.m.), performance ($P = 0.0261$; $\text{median}_{2016} = 77.5\%$, $\text{median}_{2017} = 82\%$), and sleepiness ($P = 0.0370$; $\text{median}_{2016} = 7.0$, $\text{median}_{2017} = 6.0$), emerging as significant factors between years (Fig. 3). None of the other variables, including school, sex, depression index, and chronotype, emerged as significantly different between years in the final models or any of the other models tested.

Attendance has been shown to improve and tardiness to decrease with later school start times in other school districts (*14*). We tested whether the later school start in 2017 improved attendance and punctuality by comparing the percent of absents and tardies among all students in the school in first period for each year in each school separately. Whereas Roosevelt High School (RHS) showed no difference between years, students in Franklin High School (FHS) had significantly fewer tardies and absentees in 2017 than in 2016 (Fig. 3, C and D). Notably, FHS has many more economically disadvantaged students (88%) and ethnic minorities (68%) than RHS (31 and 7%, respectively).

DISCUSSION

We show that a delay in the high school start times from 7:50 to 8:45 a.m. had several measurable benefits for students. The change led to a significant lengthening of daily sleep of over half an hour. There is evidence that adolescents in most industrialized societies do not achieve the recommended approximately 9 hours of daily sleep during school days (*5*, *15*), which is consistent with estimates that in the past 100 years, sleep has shortened by about 1 hour in children (*16*). Our study demonstrates a lengthening in the median daily sleep duration from 6 hours and 50 min to 7 hours and 24 min, restoring the historical sleep values present several decades before evenings within brightly lit environments and with access to light-emitting screens were common among teenagers. These results demonstrate that delaying high school start times brings students closer to reaching the recommended sleep amount and reverses the century-long trend in gradual sleep loss.

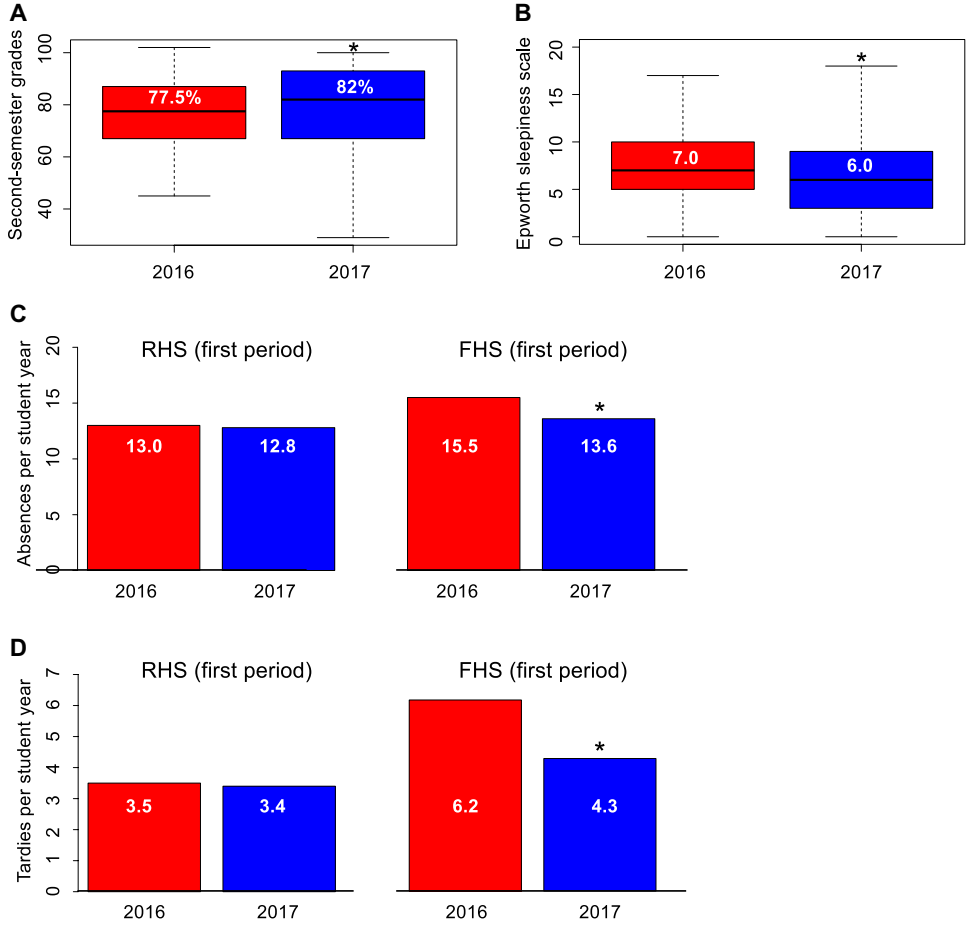

Fig. 3. Delayed school start times are associated with higher grades, reduced sleepiness, and improved attendance and punctuality. (**A** and **B**) Box plots of student performance and daytime sleepiness. Generalized linear models indicated that student performance, as measured by second-semester grades, was significantly higher (*$P = 0.0261$), whereas daytime sleepiness was significantly lower (*$P = 0.0370$) in 2017 than 2016. First-period absence (**C**) and tardy (**D**) data were compared between years using a χ^2 test. Students from FHS but not from RHS had a significant reduction in absences and tardies (*$P < 0.0001$) in 2017 as compared to 2016. Numbers within boxes in (A) and (B) represent medians, and numbers in bars in (C) and (D) represent absolute value.

We also show that the later school start time is associated with a better alignment of sleep timing with the circadian system (reduced social jet lag), reduced sleepiness, and increased academic performance. Although it is highly likely that increased sleep was the cause for reduced sleepiness, it is much harder to attribute causality for 4.5% higher grades on increased sleep; nevertheless, it is certainly reasonable that students who are better rested and more alert should display better academic performance. Last, the later school starts led to an increase in punctuality and attendance that, remarkably, was only evident in the economically disadvantaged school. Obviously, attending school and arriving on time to school is beneficial for learning, and this result suggests that delaying high school start times could decrease the learning gap between low and high socioeconomic groups. Other studies have shown impacts of later school start times that are consistent with our findings [see reviews in (*14*, *17*)]. However, to our knowledge, ours is the first report to show that an across-the-district change in school start times results in a significant increase in daily sleep measured objectively with actimetry. Carskadon and colleagues (*18*) measured sleep in a group of high school students in one Rhode Island School District that transitioned from a 08:25 a.m. start time in nineth grade to a 7:20 a.m. start time in 10th grade and confirmed a shortening of daily sleep of approximately 20 min, which was also associated with a delay in the circadian phase and increased sleepiness. A recent study showed that a delay of 45 min in start times in one all-girls high school in Singapore resulted in a modest, almost 10 min, lengthening of daily sleep (*19*). This small change in sleep duration, in comparison to our study, could be related to several differences in the study design: (i) the authors performed the study on female students and within a larger range of ages and school grades; (ii) the study was performed longitudinally on the same students before and after the school start change, which not only introduces an age difference but can also be associated with changes in schedule; and (iii) sleep parameters were based on a single week of recordings and on less recorded days per student. The nature of our pre-/post-study prevents us from having a control group. However, in an intervention study with middle school students, in which the starting time was delayed by 1 hour for a week, students gained nearly 1 hour of daily sleep compared to themselves under the normal schedule or to nondelayed controls (*20*). The Seattle school start time delay of 55 min did not result in a gain of 55 min of sleep, suggesting that after a year—as opposed to an acute change lasting for 1 week—students may delay their bedtimes, indicating that there may be other factors that are keeping teens awake in the evenings of school days. Delayed school start times should be paired with advice on sleep hygiene, including preventing the increasingly pervasive use of screens late in the evening that is known to delay sleep onset (*21*). Given the widespread negative effects sleep deprivation has on adolescent physical and mental health, our study points to the value of a measure such as delaying the school start time toward improving teenage sleep and, in turn, health and academic outcomes.

MATERIALS AND METHODS

Data collection

Activity, light, and sleep data were collected using Actiwatch Spectrum Plus wrist activity monitors. Watches were programmed to collect data in 15-s epochs for 2 weeks (14 days), and students were instructed to press a marker button on the watch each time they went to sleep and woke up. Philips Actiware (version 6) software was used to construct actograms and determine sleep intervals. Activity and light data were exported and analyzed separately for mean waveforms using R Studio and Prism. Students also completed a daily retrospective online diary, which included questions about sleep onset, offset, how they were awakened, if they took any naps, if they removed the watch, and a place for text comments. Diary information was used in the data cleanup procedure (described below) to validate the sleep bouts automatically determined by Philips Actiware.

Chronotype, daytime sleepiness, and mood were measured by a one-time completion of the respective surveys located in the same portal as the daily diary. Chronotype was assessed using the Horne-Östberg Chronotype Questionnaire (*10*) and the Munich Chronotype Questionnaire (*9*). Daytime sleepiness was measured using the Epworth Sleepiness Scale. Mood was measured using the BDI-II. Students who scored higher than 20 on the BDI-II were contacted by their teacher and reminded about access to mental health resources.

Student demographics, including sex, race, birthdate, commute time to school, and mode of transportation, were collected via a paper demographic survey handed out in class. Students were also given the opportunity to disclose any sleep disorders and/or scheduled responsibilities (work, child care, etc.) that might affect the data. In addition, students from the 2017 cohort were asked to disclose any school activities that were scheduled for before school as a result of the delayed start times; the number of cases was small and was not considered separately in the statistics. Second semester grades for the students included in the sleep study were provided by the teachers whom we partnered with for this study. These represent absolute (not normalized) grades and could carry an implicit bias from teachers who could have been for or against the school time change. Last, global attendance data for each school were provided by Seattle Public Schools.

Data were collected over the course of 6 weeks during the Spring of 2016–2017 in 2-week rounds. Students from the first period participated in data collection for the first 2 weeks, second period for the following 2 weeks, and third or fourth period for the final 2 weeks. Students in each round were given the same instructions. The data were stripped of all identifying information upon collection. At the end of the semester, the data were returned to the students for an in-class learning exercise on research methods, data interpretation, and the relationship between sleep and their lifestyles.

Participants

The first cohort of students was selected to participate from the first three periods of two sections of 10th grade Biology at RHS and one section from FHS in 2016. The second cohort of students was selected from the same grade, classes, and schools and during the same time of the year but in 2017, when the new school start time had been in effect for 7 months. Each section was taught by a separate instructor but instructors remained the same between years, and course credit was given for participating in the data collection as an in-class learning exercise. While all students were assigned to complete the online diary and surveys, watches were assigned to a subset of each section with the help of the instructors due to resource constraints. This assignment was designed to represent gender and underrepresented minorities in each class (table S1). Informed assent and consent were obtained from the students and their parents, respectively.

Inclusion criteria

Activity, light, and sleep data were segregated by school day nights (Sunday night to Thursday night) and nonschool day nights (Friday night, Saturday night, or the night before one holiday, Memorial Day) for analysis. School and nonschool days were treated separately. If a student was missing one or more nonschool nights (out of four), then the nonschool nights for that student were not included in the analysis. If a student was missing 5 or more school nights (out of 10), then the school nights for that student were not included in the analysis. Students missing both one or more nonschool night and five or more weekday nights were removed entirely from the analysis.

Activity data

Raw activity data for each subject were binned in 10-min intervals and then averaged across either school or nonschool days, giving each subject two averaged 24-hour activity profiles. Individual profiles were then used to construct activity waveforms for school and nonschool days in 2016–2017 (Fig. 1). Waveforms were analyzed using a two-way ANOVA with year and time of day as factors, followed by Sidak's multiple comparisons.

Light data

Light exposure data were analyzed using the "white light" reading supplied by the Actiwatch. Light readings are subject to occlusion by sleeves, and they are also attenuated when the light source is off-axis [50% attenuation at 50° to 60° and near-complete attenuation at 70° to 80° (*11*, *12*)]. It is therefore conceivable that illuminances above 100 lux at normal arm position could result in momentary readings of 0 to 10 lux. Figure S1 shows that this does happen; even during the middle of the day, when an exposure to illuminances lower than 1 lux is extremely unlikely, a large proportion of individual readings are very low. In addition, levels of illuminance <~1 lux resulted in a greatly increased measurement error with these devices (*11*, *12*). Therefore, we excluded readings during which the subject was asleep, the watch was off wrist, or illumination was below 1 lux. Raw data for the same students that met inclusion criteria for activity were binned in 10-min intervals and processed for waveforms (Fig. 2).

The >1 lux criterion does not completely preclude momentary incorrect measurements. However, while Actiwatches can underestimate illuminance levels by more than an order of magnitude, they are not known to do the opposite, i.e., provide artifactually high illuminance values. Therefore, the times of first and last exposure to a given light threshold represent a more meaningful measurement than the mean of the illuminance reading. While this measure does not fully describe the illuminance over the 24-hour period, it provides a direct measurement of how long a subject experienced light levels above a physiologically interesting value. Figure S2 portrays the difference between the probability of the mean of all readings within a 10-min interval being above 50 lux and the probability of a single reading being above the 50 lux. Inside a given 10-min bin of a student day, three possibilities exist: (i) no measurements are >50 lux; (ii) one or more measurements are >50 lux, but the mean is <50 lux; and (iii) the mean measurement is >50 lux. Figure S2 shows that during the early morning and late evening, when a person's watch produces a single reading above 50 lux, it is more likely that the mean of the readings in these 10-min intervals will be below 50 lux than it is for the mean to be above 50 lux. Given that most subjects experience indoor light levels at this time range and the potential for inaccurately low, but not high, measurements discussed above, times of the first and last light measurements above a given threshold are a more accurate depiction than the mean illuminance reading at a given time. The former variable represents a better proxy for the overall window of time during which the subject experiences physiologically relevant levels of artificial light. This analysis could potentially use any threshold; the choice of 50 lux was based on the estimated threshold for the inhibition of melatonin release.

The time of first and last exposure to a given light intensity threshold was calculated separately for each student day. We report the mean value of first and last exposure to a 50-lux threshold intensity separately for school days and nonschool days for each school year (Fig. 2C). Data were analyzed by two-way ANOVA with day of week (school or nonschool) and year as factors, followed by Sidak's multiple comparisons. Light data were processed using Python v2.7.9, using the following libraries: pandas v0.22, numpy v1.14.0, and matplotlib 2.1.0.

Sleep data cleanup

For this study, we gathered three potential sources for sleep onset and offset data. The first was the watch, which extrapolated sleep intervals from the raw data using Philips Actiware software. The second were the self-report online diaries that the students were assigned to fill out every day. The third were the time stamps students could add to their recordings by pressing a button on their watch. To validate the sleep intervals, as determined by the watch software, we followed the following protocol:

1) We inspected every single actogram day by day to detect false software calls for sleep onsets or offsets. These represent obvious mistakes that can be easily detected upon inspection (fig. S3).

2) We then looked for discrepancies between the diary, or event recorder, and software calls for sleep onsets or offsets that were larger than 1 hour.

3) For all the nights in which the discrepancy was larger than 1 hour, we inspected once again the actograms on those nights to determine whether the 1 hour error was due to a software error or a student error, entering the wrong time in the diary or with the event marker.

After inspection of the actograms in step 3, we determined that 19% of the discrepancies had already been detected in step 1. Of the remaining discrepancies, 77% were caused by student error in the diary or event marker and 19% by a watch error, and on 4%, we were unable to determine the cause. This means that the error of taking the actimeter calls for sleep onset and offset after step 1 is ≤5% and four times lower than the student-generated error, which was 19.5%.

Cleaned sleep data were broken up by onset, offset, duration, and efficiency for school and nonschool days. Sleep data were then exported and analyzed using Python and R Studio. Normality was tested by (i) visual inspection of distribution histograms, (ii) quantile/quantile plots, and (iii) through the Shapiro-Wilk test. None of the variables had normal distribution, and data on Fig. 1 were presented as medians ± quartiles. Differences in sleep onset, offset, and duration for both school and nonschool days were tested using Wilcoxon signed-rank tests with a Bonferroni correction for multiple comparisons. For each student, social jet lag was calculated as the difference between mean mid-sleep on the nonschool days (after subtracting oversleep) minus mean mid-sleep on the school days (*22*). For the

Wilcoxon signed-rank tests, effect sizes were calculated by dividing the *U* statistic by the product of the *N*s (*23*).

Academic performance and attendance

Academic performance was assessed using second-semester grades from the science class that provided our pool of participants. School, academic performance, mood, chronotype, and sleepiness values were scaled and tested via generalized linear models using a binomial family with year as the dependent variable, testing the hypothesis that years differed on the basis the other variables. Models included a single sleep variable (onset, offset, duration, and efficiency) at a time to avoid multicollinearity. Multiple models were tested, and the model with the lowest Akaike information criterion value was selected as the model of best fit. The final model included school, academic performance, mood, chronotype, sleepiness, and weekday offset. Ethnicity was not tested as a variable per se, but the two schools differ widely in their ethnic backgrounds (table S1). There were no sex differences between years or schools. There is no consensus on how to calculate the effect size for each variable that emerges as significant with generalized linear models; instead, we present the medians for each year, as the data were not normally distributed.

Attendance data were provided by the school district and contained the average number of tardies and absences per student by period for both schools in the study for 2016–2017. Predicted absentee and tardy data for 2017 were calculated on the basis of the rates from 2016, adjusted for changes in enrollment, and assessed using a χ^2 test. This analysis was only performed for the "first period," namely, the first scheduled hour of class in the morning.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/12/eaau6200/DC1

Table S1. Demographics of students in each of the high schools included in the study.

Fig. S1. Probability of a light measurement (among all individuals recorded) being below a threshold (X in legend) throughout the day.

Fig. S2. Probability distribution of light measurements across all watch data from Seattle high school students in 2016 and 2017.

Fig. S3. Representative actogram of a student in which the Actiwatch algorithm for sleep offset detection missed a sleep offset (white arrow).

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Supplementary Materials for

Sleep*more* in Seattle: Later school start times are associated with more sleep and better performance in high school students

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This PDF file includes:

Supplementary Materials:

Table S1. Demographics of students in each of the high schools included in the study.

School	**RHS**		**FHS**	
Year	**2016**	**2017**	**2016**	**2017**
Students	51	41	41	41
% Female	53	49*	56	58
% White	76	75	2	19
% Asian	10	5	54	46
% Hispanic	6	5	7	7
% African American	8	5	32	22
% Unknown/other	0	10	10	10
Age (mean ±SEM)	16.08 ± 0.05	16.27 ± 0.08	16.13 ± 0.05	16.13 ± 0.06

*Percent calculated over a total of 37 students, 4 students did not disclose sex.

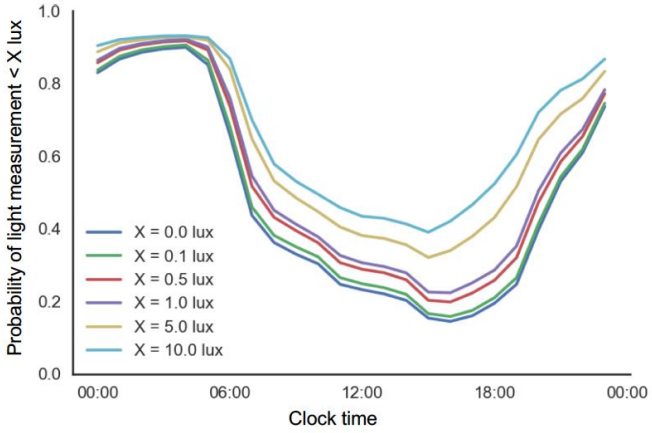


Fig. S1. Probability of a light measurement (among all individuals recorded) being below a threshold (X in legend) throughout the day. Probabilities are calculated over all student data irrespective of day of the week or year.

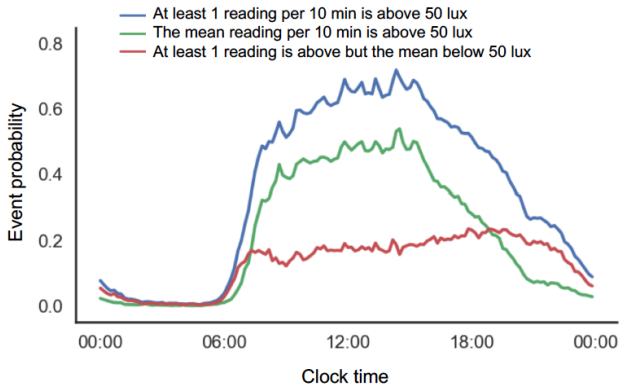


Fig. S2. Probability distribution of light measurements across all watch data from Seattle high school students in 2016 and 2017. Lines show the probability of a 50lux illuminance threshold criterion being met. Probabilities are calculated separately for each 10min bin across student-days data. Blue line shows the probability of at least a single measurement being above 50 lux. Green line shows the probability of the mean of the measurement within the 10min bin being above 50 lux. Red line represents the blue line minus the green line: the probability that at least one reading is above 50 lux but the mean reading is below 50lux.

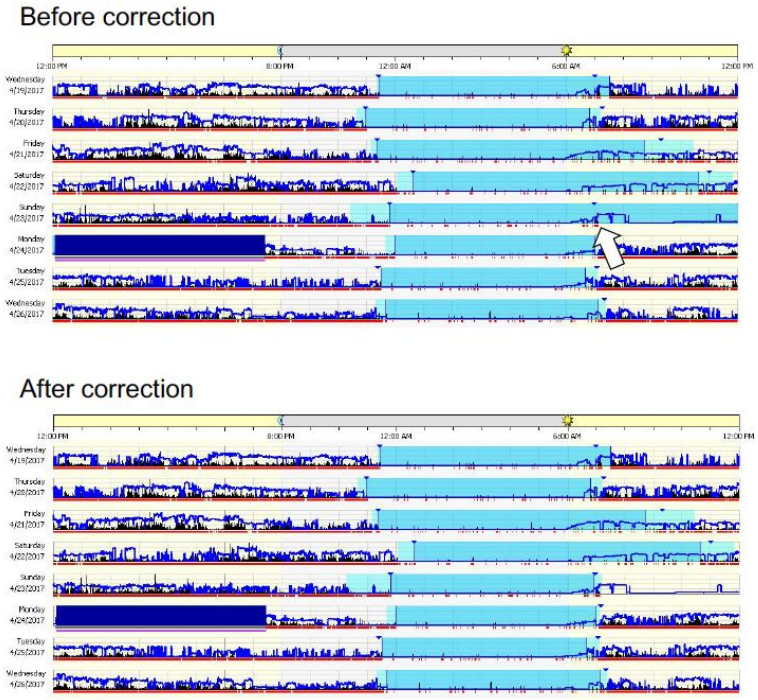


Fig. S3. Representative actogram of a student in which the Actiwatch algorithm for sleep offset detection missed a sleep offset (white arrow). Each row represents a 24h day and successive days are stacked vertically. The same actogram is shown after the error was corrected by the experimenter. Light blue rectangles represent sleep episodes, the dark blue rectangle represents a time window when the Actiwatch was off-wrist. Black markings on each day represent activity intensity, blue contours represent light exposure to blue light. Top bars represent natural day (yellow between sunrise and sunset) and natural night (gray between sunset and sunrise.

ABSTRACT

When students move from high school to university settings, they undergo many lifestyle changes. One significant change is an increased control over their sleep schedules, which can have implications for their mood, academic performance, and general well-being. Sleep timing is partially controlled by the biological clock housed in the suprachiasmatic nucleus (SCN), which receives information from the natural environment to time behaviors like sleep and arousal. The most important natural signal for the SCN is light, which advances the clock in the morning and delays the clock in the evening. The amount of natural light available can vary throughout the year, especially in more northern and southern latitudes, which may alter the timing of the biological clock throughout the year. On the other hand, access to electrical lighting may dampen these seasonal rhythms as individuals in industrialized societies are not as reliant on the natural solar cycle. In order to assess the interaction between seasons, sleep timing, academic performance, and other lifestyle outcomes we paired with Biology courses at the University of Washington to collect data from undergraduate students covering four academic quarters (Fall, Winter, Spring, Summer) over the course of three years. Results show a significant delay in sleep timing in the Winter compared to the Summer, providing strong evidence for the presence of seasonal rhythms in humans living in industrial societies. In addition, we found that later bedtimes were associated with worse academic performance and a potentially indirect relationship between sleep duration, mood, and academic performance.

INTRODUCTION

While the exact purpose of sleep is still being debated[1,2], it is clear that sleep is critically important for learning and memory[3] in two major ways: sleep after learning helps consolidate memories for long term storage, and sleep preceding learning allows the brain to efficiently process new information. Insufficient sleep has been shown to impair working memory[4], a critical function that allows the brain to process and sort new information during wake. Impairing working memory can reduce the ability to form important short-term memories and thus decrease overall learning. For example, subjects who were sleep deprived performed worse on a paired word association test than subjects who were not[5]. Furthermore, acute

insufficient sleep after the acquisition of information impairs the movement of memories from short term to long term storage in the brain. For instance, individuals deprived of slow-wave sleep (SWS) or rapid-eye movement (REM) perform worse on procedural and declarative memory tasks, respectively[6]. In another study, subjects who were sleep deprived after learning a word association task performed significantly worse compared to subjects that were allowed to sleep immediately after[5].

In 2015, the National Sleep Foundation updated their sleep recommendations and introduced a new category for young adults (18-25), advocating for 7-9 hours of sleep per night due to the critical importance of sleep for physical and psychological health[7]. However, research has shown that many college students are not getting enough sleep or tend to have poor sleep quality[8]. Insufficient sleep can have a myriad of negative consequences. A recent survey of undergraduates found that over 60% of students rely on stimulants to maintain alertness during the day, nearly 12% abuse alcohol as a sleep aid, and 16% have reported falling asleep while at the wheel of a motor vehicle[9]. Poor sleep has also been associated with elevated depression levels[10] and habitual short sleep has been linked to an increase in all-cause mortality in the general population[11].

Universities offer a unique opportunity to test the effect that sleep has on learning and memory in a real-word setting. The primary responsibility for college students is the efficient acquisition of knowledge on a variety of topics. In addition, for many students attending college it represents the first time when they can set their own sleep schedules. This is in contrast to other populations of students, for example those attending high or middle school, where sleep and activity schedules are dictated to a much greater extent by a parent or guardian.

Previous work has suggested that academic performance may be influenced by sleep in college students, but results have been inconsistent. A recent study investigating the influence of chronotype, a term for the preferred time of activity in humans, found that university students who reported a preference for early activity had higher GPAs that their later peers[12]. A similar study in Dutch high school students also found an effect of chronotype, but only in STEM courses and not the humanities, and a weak effect of sleep duration on academic performance[13]. On the other hand, two studies that used sleep diaries[15] or timestamped

activity on the main online system for student academic activity[16] found that later sleep onsets, sleep variability, and social jetlag (the difference in the time of mid-sleep between weekdays and weekends) were correlated with course performance in college. Similarly, a 2013 study that investigated sleep patterns in medical students found that the time of mid-sleep was predictive of grade[14]. Yet another study that tracked sleep via self-report diaries in undergraduate students found that sleep variability was correlated with performance, but only after extending the study to 30 days instead of the initial 14[15]. Unfortunately, the variety of methods employed in these studies to track sleep and assess performance make it difficult to conclude what aspects of sleep are directly or indirectly contributing to academic success.

In addition to the role nightly sleep has on academic performance, little is known how the changes in seasons affect university students. With the exception of populations that live close to the equator, different seasons come with changes in the amount of natural light, or photoperiod. Light is the strongest signal for the human circadian system[16–18], which partially controls the timing of sleep[19]. While artificial light has allowed humans to live partially independent of the natural photoperiod[20,21], there is some evidence that even in industrialized societies human sleep and activity changes across seasons[22,23]. For example, one of the most commonly recognized impacts of shortened photoperiod during the winter is seasonal affective disorder (SAD), a seasonal depression attributed to the shortening of natural daylight at higher latitudes[24]. While one study of SAD in college students from New England did find a seasonal effect, it was only found in students who moved to college from areas with a southern latitude[25]. As a result, how changes in season affect either sleep or mood in college students is still largely unknown.

Previous work in our lab has found that sleep diaries are unreliable[26]. Thus, we apply automatic wrist-worn data loggers to create a highly objective, high resolution sleep database spanning 3 years and including over 500 subjects. In this study we use that database to address three main questions. First, is there evidence of changes in sleep behavior and affect across seasons? Second, what aspects of sleep impact academic performance? Third, what variables influence how students sleep? By combining objective measures of sleep with information about chronotype, depression, daytime sleepiness, demographics, and GPA we hope to further

our understanding of the complex relationships between sleep, mood, and performance in college undergraduates at the University of Washington, but also to stablish basic principles that underly the relationship between sleep and different aspects of college life.

METHODS

Data collection

Students wore an Actiwatch Spectrum Plus® (Phillips Respironics) programmed to sample activity and light in 15-s epochs on their non-dominate wrist for two weeks. In addition, students were instructed to press an indicator button on the watches each time they went to sleep or woke up. Philips Actiware (version 6.0.9) software was used to construct actograms and determine sleep intervals. Students also completed a daily retrospective online diary, which included questions about sleep onset, offset, how they were awakened, if they took any naps, if they removed the watch, and a place for text comments. Marker information was used in the data cleanup procedure to validate the sleep bouts automatically determined by Philips Actiware. Sleep diaries were consulted in the event of a conflict between the watch and markers.

Chronotype, daytime sleepiness, and mood were measured by a one-time completion of surveys located in the same portal as the daily diary. Chronotype was assessed using the Horne-Östberg Chronotype Questionnaire[27]. Daytime sleepiness was measured using the Epworth Sleepiness Scale, Mood was measured using the BDI-II. Students who scored higher than 20 or answered with a 1 or a 2 (low to moderate threat of suicide) on question 9 on the BDI-II were contacted by their instructor and reminded about access to mental health resources.

Demographic information, including sex, race, ethnicity, birthdate, first generation status, and educational opportunity status were collected from the University of Washington registrar. Student GPA for the biology course in which the study was run was collected from the registrar after the quarter ended along with the individual GPAs of all courses from the same quarter. Academic performance was calculated by averaging the GPA from all courses. To investigate academic performance, demographic data were collapsed into four main categories: Sex, Underrepresented Minority (URM) status, First Generation (FG) status, and Educational

Opportunity (EOP) status. There are four levels for race/ethnicity: Non-URM (White and Asian students), URM (African-American, Latino/Latina, Native American, and Pacific Islander/Native Hawaiian), International students, and not identified. First generation status was identified as any student who was the first in their family to attend or anticipate graduating from college. EOP status was restricted to students who are identified as EOP1 or EOP3 by the registrar. Other groups with special categorical recognition by the registrar, such as student athletes or staff, were not recognized as EOP for this manuscript.

Participants

Data acquisition was done in partnership with biology faculty teaching either one of two courses: Biology 220 (the third class in an introductory series) or Biology 418 (an upper level circadian biology course). In exchange for providing access to a student population, each quarter the data were returned to the students as a part of a learning exercise on sleep, academic performance, circadian rhythms, and the process of science. Students received course points for their participation in the data acquisition and discussion, and at the end of the quarter they were given the option to anonymously contribute their data to the larger study. The number of course points for participation were decided by the professor leading each course.

Data were collected in two-week intervals. In quarters where the number of participants exceeded the number of available watches, students were divided into cohorts with one cohort participating in data collection followed by a second cohort, until all possible subjects within that quarter participated; in some of the quarters the class size was so large (>300) that only a sample of students wore a watch. Data were collected non-sequentially from each of the four academic quarters at the University of Washington (Fall, Winter, Spring, Summer) between the Fall of 2015 and Spring of 2018. Table 1 summarizes the number of subjects, collection rounds, and dates of data collection for each quarter represented.

Sleep Data Cleanup

Data were cleaned using the same protocol described in Dunster et al., 2018. Sleep data were segregated into School nights (Sunday-Thursday) and Free nights (Friday and Saturday). The only exceptions were for national holidays observed by the University of Washington, in which case the night preceding the holiday was classified as a Free night. During the Fall some subjects collected data during the change for Daylight Savings Time (DST). Due to the effects DST has on shifting the clock, the night of DST and the 3 subsequent nights were discarded from the analysis.

Testing the Difference Between Quarters

Average School Night Onset, Duration, Social Jetlag, Mood, and Sleepiness were tested for quarter (season) effects. Kruskal-Wallis tests followed by Dunn's Test of Multiple Comparisons Using Rank Sums were used due to the non-normal distribution of the data. Post-hoc tests were only used on variables that showed a significant effect from the Kruskal-Wallis test and analyses were restricted to non-academic variables due to the differences between class structures.

Academic Performance Linear Model Selection

We used a linear model selection paradigm to determine what variables contributed to academic performance in our population. For each model, the outcome of academic performance as measured by the average GPA for all classes across the quarter. Due to the inherent, fundamental differences between upper and lower level courses, data for Biology 220 and 418 could not be analyzed together. For this study, we report the results from Biology 220 alone. To avoid issues of multicollinearity, four different models were fit to the data. Each of the four models began with the same set of explanatory variables (see below) but contained a different demographic variable (Sex, FG, URM, EOP). Once a model was run, one of the explanatory variables that did not significantly contribute to the outcome was removed and a second model was run. To assess the impact of the missing variable, a likelihood ratio test (ANOVA) was run and the AIC values of the two models were compared. If there was no

significant difference between the models as shown by the results of the ANOVA and if the AIC value for the second model was lower, model selection continued. A third model was tested, again removing a non-significant variable, and was tested against the second model using an ANOVA and comparing the AIC values. This process continued until there were no more non-significant variables to remove, or the results of the ANOVA and AIC indicated that removing a variable resulted in a weaker model. Once this process was complete for all four student subpopulations the results were compared.

With the exception of the demographic variable, each model began with the same explanatory variables. Average school night sleep duration and social jetlag were included due to their prominent focus in the sleep and circadian fields[7,28]. First, many people measure the health of their sleep by the amount of sleep they get in keeping with the recommendations from the National Sleep Foundation. Second, as a measure of the changes in sleep patterns between school and free days, social jetlag is a common way to represent the relative stress the circadian system is under on a given week. Additionally, social jetlag is calculated from the midpoint of sleep, not sleep duration, so both sleep variables could be included without incurring issues of multicollinearity. The models also included the individual's chronotype as measured by the Horne-Ostberg questionnaire and the scores from Beck's Depression Index and the Eppwroth Sleepiness Scale. Finally, Quarter and Year were included to account for any potential differences in the sampling of the data.

Preliminary analysis indicated that Average School Night Bedtime may have had a significant effect on academic performance. As a result, one additional set of models was selected with Average School Night Onset instead of Average School Night Duration as an explanatory variable. Due to the tight association between Average School Night Onset and chronotype, the results of the Horne-Ostberg questionnaire were not included in the model.

Sleep Outcome Model Selection

To determine what variables may be contributing to differences in student sleep, a similar method of model selection was implemented to the one described above. In this case, two sleep variables were selected as the outcomes: Average School Night Onset and Duration.

There was no *a priori* reason to believe that FG, URM, and EOP status may have a significant effect on sleep. Thus, initial models for both sleep outcomes included Quarter, Year, Sex, Ethnicity, Chronotype, and Mood. Ethnicity was selected over Race as an explanatory variable because ethnicity had fewer categories and thus each category contained a larger and more meaningful sample size. For both sleep outcomes, model selection proceeded in the exact same manner as above using backward selection while comparing the ANOVA and AIC values for each subsequent model until a best fit was determined.

Testing the Effect of Sleepiness

Given the interdependency of nightly sleep on sleepiness, sleepiness was not included in the linear models for Average School Night Onset or Duration. Instead, the relationship between sleepiness and Average School Night Onset and Duration were tested using separate correlational analyses. Due to the non-parametric nature of the data, we used Kendall's tau with a corrected threshold of $p < 0.025$ for significance.

RESULTS

College Students Show Seasonal Changes in Daily Sleep and Mood

Figure 1 presents changes in sleep variables across seasons. A Kruskal-Wallis test yielded an effect of quarter for Sleep Onset during school days (Kruskal-Wallis $X^2_{(3)} = 18.98$, $p < 0.001$) (Fig 1A). Post-hoc analysis using Dunn's Test of Multiple Comparisons revealed that during the Winter quarter, Sleep Onset was significantly later than both Spring ($Z = -3.87$, $p < 0.001$) and Summer ($Z = -3.07$, $p = 0.012$) quarters, but not Fall quarter ($Z = -1.41$, $p = 0.935$). Sleep Onset during Fall quarter was also significantly later than Spring quarter ($Z = 2.73$, $p = 0.037$) but no other quarter. Sleep Offset also showed a main effect of quarter (Kruskal-Wallis chi-squared = 9.1311, df = 3, $p = 0.028$) (Fig 1B). A post-hoc analysis revealed that once again Winter quarter was significantly different from Spring quarter ($Z = -2.68$, $p = 0.044$). In contrast, there was not an effect of quarter on Sleep Duration (Kruskal-Wallis chi-squared = 2.7563, df = 3, $p = 0.431$).

Mood and sleepiness scores also showed a significant effect of season (Kruskal-Wallis $X^2_{(3)} = 10.331$, $p = 0.016$ for mood and $X^2_{(3)} = 8.9441$, $p = 0.030$ for sleepiness) (Fig 1C-D). Post-

hoc tests for mood revealed that Spring quarter was significantly lower than Summer quarter ($Z = -2.67$, $p = 0.044$) and was nearly significantly lower than Winter quarter ($Z = -2.51$, $p = 0.071$). For sleepiness, multiple comparisons revealed Winter sleepiness to be significantly higher than Summer quarter ($Z = -2.85$, $p = 0.026$). There was not an effect of quarter on Chronotype (Kruskal-Wallis $X^2_{(3)} = 2.0226$, $p = 0.568$).

Chronotype, sleep onset, sleepiness and mood predict academic performance

We used four linear models with average quarterly GPA as the outcome variable to assess what factors contribute to academic performance (Fig 2). At the University of Washington class grades are assigned on a 4.0 scale, however, data from the registrar is reported on a 40-point scale (retaining the scalar nature of the data without a decimal point). Each of the four models began with the same set of explanatory variables (Quarter, Year, Sex, Chronotype, Average School Night Duration, Social Jetlag, Depression Score, Sleepiness Score) but contained a different demographic variable (Sex, FG, URM, EOP) to avoid issues of multicollinearity. In three of the four cases, a single model of best fit emerged ($F_{(3, 169)} = 7.379$, $p < 0.001$) with Chronotype ($\beta = 0.174$, $p = 0.007$), Mood ($\beta = -0.207$, $p < 0.001$), and Sleepiness ($\beta = 0.129$, $p = 0.041$) as significantly associated with academic success (Fig 2). For these models, Sex, URM status, EOP status, Quarter, Year, Social Jetlag, and Average School Night Duration did not emerge as significantly associated with academic success. In our fourth model ($F_{(6, 166)} = 4.859$, $p < 0.001$) FG status emerged as trending toward significant ($p = 0.08$).

Due to the close relationship between Sleep Onset and Chronotype ($F_{(1, 371)} = 126.8$, Adjusted $R^2 = 0.253$, $p < 0.0001$) (Fig. S1) and the derivative nature of Sleep Duration on Onset and Offset, another set of models was selected with Sleep Onset replacing Chronotype as a potential explanatory variable. Once again, in three of the four cases a single model of best fit emerged ($F_{(3,169)} = 6.953$, $p < 0.001$) with Average School Night Onset ($\beta = -0.181$, $p = 0.014$) (Fig 4), Mood ($\beta = -0.22$, $p < 0.001$) and Sleepiness ($\beta = 0.139$, $p = 0.03$) emerging as significantly associated with academic performance. In this model, Sex, URM status, EOP status, Quarter, and Year were not significantly associated. In a fourth model ($F_{(4, 168)} = 6.667$, $p < 0.001$) FG status emerged as significantly associate with academic success ($p = 0.023$).

Chronotype, Sex and Ethnicity Predict Sleep Timing on School Nights

To determine what variables were associated with school night sleep, we used two different linear models for Average School Night Duration and School Night Onset. The single model of best fit for Duration ($F_{(11, 338)} = 6.109$, $p < 0.0001$) included Sex ($p = 0.004$, $Mean_{Male}$ = 6:47 h, $Mean_{Female}$ = 7:05 h) (Fig. 3B) and Mood ($\beta = -0.112$, $p = 0.02$) (Fig. 4). Additionally, Ethnicity was a significant predictor of Duration with Asian and African American students sleeping significantly shorter than White students ($p < 0.001$, $Mean_{White}$ = 7:13 h, $Mean_{Asian}$ = 6:36 h, $Mean_{African\ Am.}$ = 6:30 h). No other ethnicities emerged as significant.

The model of best fit for Onset ($F_{(14, 336)} = 21.61$, $p < 0.0001$) included Sex ($p < 0.001$, $Mean_{Male}$ = 1:07 AM, $Mean_{Female}$ = 12:48 AM) (Fig 3A) and Chronotype ($\beta = -0.457$, $p < 0.0001$). Quarter also emerged as significant, with Winter ($p = 0.003$), Spring ($P < 0.001$) and Summer ($p < 0.001$) onsets all significantly different than Fall quarter, which further supports the results of the seasonal changes from the Kruskal-Wallis test (Fig 1). Finally, Ethnicity was a significant predictor of Onset; both Asian ($p = 0.001$, Mean = 1:17 AM) and International ($p < 0.001$, Mean = 1:38 AM) students went to bed significantly later than White students (Mean = 12:32 AM). No other ethnicities emerged as significant.

Later Onset and Shorter Sleep Associated with Increased Sleepiness

To assess the relationship between self-reported sleepiness and Average School Night Onset and Duration we used two separate non-parametric correlational tests with a corrected threshold of $p < 0.025$ for significance. Both Average Weeknight Onset ($z = 3.25$, $p = 0.001$, tau = 0.121) and Duration ($z = -3.90$, $p < 0.0001$, tau = -0.145) were significantly correlated with daytime sleepiness. Specifically, later onset and shorter sleep duration were correlated with increased daytime sleepiness.

DISCUSSION

In this study we sought to combine objective measures of sleep with information on depression, sleepiness, academic performance, and demographic information to answer three main questions. First, are there measurable differences in sleep and affect among quarters? Second, which sleep variables contribute to academic performance? Third, what variables influence student sleep patterns? The results show quarterly differences in sleep onset, offset, mood, and sleepiness, suggesting possible seasonal rhythms in college undergraduates. Using linear models, we found that sleep onset, chronotype, mood, and sleepiness were associated with academic performance. We also report significant differences in sleep onset and duration by sex and mood. Finally, we found significant correlations between sleep onset and duration and reported sleepiness. Together, these results represent an important step in understanding the complex relationship between sleep, mood, and academic performance in college students.

Seasonal Rhythms

Seasonal rhythms of sleep in humans can be difficult to identify in industrialized societies using field studies[23,29]. Electricity, and more specifically electric lighting, have allowed humans to keep relatively similar patterns of sleeping and waking regardless of the time of year[20,21]. In this study we show strong evidence for the effect of season, and more specifically Winter, on our student population. Both weekday bedtimes and rise times were significantly delayed during the Winter quarter (Fig 1A-B). Congruently, average depression and sleepiness were the highest during the Winter quarter (Fig 2C-D). However, even with these changes in timing and affect, average weekday sleep duration did not significantly change across quarters, indicating that while students are still sleeping the same amount the timing of their sleep is being influenced by the environment.

With a latitude of ~47°N, Seattle is one of the northernmost major cities in the continental United States. As a result, the photoperiod across years changes drastically from nearly 16 hours of daylight during the summer solstice to 8 hours of daylight during the winter solstice. This reduction in daylight hours causes humans living in highly urbanized regions to be

exposed to weaker artificial light in the mornings and also extend their evening artificial light exposure[21]. Morning light is typically responsible for advancing the clock while evening light is responsible for delaying it[30]. By reducing light exposure in the mornings and extending light exposure in the evenings, the human circadian system receives a weak advance signal and a relatively stronger delay signal to the circadian system, thus delaying the clock compared to the natural photoperiod. Indeed, that is exactly what we see in our student population with regard to their sleep timing: delayed sleep onset and offset in the Fall and Winter quarters when compared to the Spring and Summer quarters. This finding supports the general notion that the human circadian system is still highly synchronized with solar time, and that artificial light during the winter—at least in high latitudes—fails to mimic a summer photoperiod[31].

Although average weekday sleep duration did not change across quarters, students still reported being significantly more tired in the Winter when compared to the Summer quarter. Previous lab studies have shown a disassociation between core body temperature and sleep in humans during the winter[32]. As forced desynchrony studies have shown, sleep quality is highest when individuals sleep in phase with their temperature rhythm[33]. Thus, it is possible that the increase in sleepiness seen during the Winter quarter is the result of an internal misalignment between internal temperature rhythms, as well as other physiological rhythms that are heavily dependent on the endogenous circadian clock, and sleep stages that are more dependent on homeostatic regulation of sleep, resulting in lower quality sleep. It is also possible that reduced exposure to natural daylight, which is an arousing stimulus itself[34], during the Winter is contributing to increased sleepiness.

Finally, our study shows that mood varied significantly across seasons with the lowest depression scores reported in the Spring and the highest in the Winter and Summer quarters. Seasonal Affective Disorder (SAD) is a subtype of depression that peaks during the winter months and is more prevalent for individuals living in higher latitudes[24]. Evidence from college students has shown an increase in SAD symptoms during the winter, consistent with the findings of our study[25]. The fact that our students show a delayed sleep timing in the Winter quarter is consistent with decreased mood. A study of subjects using a constant routine found that those with SAD had significantly delayed circadian rhythms compared to the controls[35].

Furthermore, it found that morning light exposure significantly advanced the circadian rhythm. It is possible that the delayed sleep rhythms and the increase in depression observed during the winter in our students are both related to a delayed phase due to the lack of natural morning light.

Although depression due to SAD typically subsides during the spring and summer months[18], other studies have found summer can also be associated with depressive symptoms[36]. However, it is difficult to attribute the elevated summer depression observed in our study to SAD given the early sunrise times in the summer months in Seattle. It is possible that the differences in mood are an artifact of the study design. Data from the spring comes from predominantly upper-level Biology students while data from the other three quarters comes from predominantly introductory-level Biology students. When the data from the lower-level biology course are analyzed alone, there is no longer a difference of quarter in mood scores ($p = 0.8378$). Perhaps upper-level students have more stable mood because of their college experience, while introductory-level students are still adjusting to the new challenges that college presents. Finally, it is important to note that the Beck Score of depression does not represent a professional diagnosis of depression or seasonal affective disorder.

Academic Performance

The relationship between sleep and memory have long been established in human and animal studies[3]; however, what aspects of sleep are most important for academic success, which relies heavily on learning and memory, in field studies has not been as clear. While organizations like the National Sleep Foundation (NSF) advocate the importance of sleep duration[7], data has shown that sleep duration may only have a weak effect on academic performance[13,37]. Consistently, we found no association between average weekday duration and grades in any of the models tested. Based on these data, it would be tempting to conclude that it does not matter how much a student sleeps each night. However, given the negative effect of sleep deprivation on learning and memory[4] this may be premature. The average weekday duration (6.97 +/- 0.96 hours) for our sample was at the minimum of 7 hours recommended by the NSF and less than 1% of our population slept the full 9 hours

recommended. Given that previous studies have shown performance deficits in subjects sleeping 8 hours per night[38], it is possible that the majority of our subjects have chronically decreased academic performance. Furthermore, many courses at the college level curve final grades, which may reduce the inter-student variability and hinder our ability to measure the effects of sleep deprivation on course performance. In other words, if everyone is performing suboptimally in a course due to sleep deprivation, no one will appear to be performing poorly after grade curving.

Chronotype and average weekday sleep onset were both significantly associated with academic performance (Fig 2A, C). Specifically, students with later chronotypes and later bedtimes had lower GPA's than their earlier counterparts. While not interchangeable, chronotype and sleep onset have a strong interdependence (Supp. Fig. 1) and will be considered together for this discussion. These results are consistent with the results from a wide variety of other field studies in high school[13,39], college[40,28], and medical students[41] which find that students with later chronotypes and/or bedtimes typically perform worse in school. One explanation for this trend is that morning type students are better prepared for classes and exams in the morning than evening type student. However, a recent study found that even when controlling for the time of day of class, the difference between chronotypes remained[12]. Another possible explanation is that later chronotypes are more sleep deprived. However, we found no correlation between chronotype and sleep duration in our sample and, as stated above, sleep duration had no impact on GPA. Thus, our results contribute to the growing body of evidence supporting a relationship between chronotype and academic performance and suggests that the negative effects of a later chronotype may related to a delayed phase of entrainment of the circadian system and the negative effects associated with it[42], but not necessarily to sleep duration itself.

Our study also shows a significant negative relationship between depression scores and academic performance (Fig 2B). The relationship between mood and academic performance is complex and multidimensional, but inability to concentrate and low motivation are both common symptoms of depression[43]. In addition, there is evidence that depression interferes with working memory[44]. This would suggest that depression is a cause of poor academic

performance. However, there is also evidence that worrying about academic performance causes depression in college students[45]. Thus, while our study further strengthens the relationship between depression and academic performance, we are unable to tease apart any causal explanations.

We found a significant relationship between sleepiness and academic performance (Fig 2D). However, counter to previous studies[41], academic performance was *positively*, although with a small effect size, associated with sleepiness in our study. In our study, students were only asked to complete the survey once rather than every day or multiple times a day. It is possible that a single measure of sleepiness was not a sufficient representation of student affect, as sleepiness can be influenced by things other than nightly sleep such as the use of stimulants. To further investigate the relationship between sleepiness and academic performance, future studies should consider multiple measures from each student with a control for time of day, stimulant use, and other potentially confounding variables.

Our study found no association between Sex, URM status, or EOP status and only a marginally significant association between FG status and academic performance. While it is possible that these results are due to our use of average GPA for all courses during the quarter rather than the GPA from the Biology course alone, it is critical to acknowledge that our linear models were run without taking previous academic success into account. As it has been shown, controlling for previous academic success is important when testing models on academic performance[46]. Future analyses of these data will include incoming GPA as a proxy for previous student success.

Nightly Sleep

The final aim of our study was to understand what factors were influencing student sleep. We observed that females went to bed earlier and slept longer than males on week nights (Fig 3). This result is consistent with other studies showing sex differences in sleep timing[47,48] that coincides with the entry into puberty. During puberty most adolescents undergo changes to their circadian system which typically results in a delay in their chronotype. Males, who have higher concentrations of androgens, typically delay their circadian systems more than

females, who have higher concentrations of estrogens and progestins[48,49]. Changes in sleep associated with puberty are supported by experiments in several animals models, suggesting they are not just as consequence of changes in the social life of adolescents[50]. While the relationship between these hormones and sleep timing is still unknown in puberty, exogenous estrogens and progestins have been shown to increase sleep duration and reduce sleep latency in females making them excellent candidates for future studies.

We also observed a negative relationship between depression score and sleep duration (Fig 4). While it is commonly assumed that depression leads to increased sleep, a recent meta-analysis of over 25,000 individuals found that both abnormally short and long sleep was associated with depressive adults[10]. In addition, a recent study surveying sleep patterns and depression in adolescents found that sleep quality worsened as depressive symptoms increased[51]. Our results suggest that, at least in college students, individuals with increased depression sleep less. Importantly, sleep and depression are clearly interdependent and the correlation we find cannot define any causation. It is conceivable that our data reflect the influence short sleep—the vast majority of our students are sleep deprived—on mood. Of note, while we did not find a direct relationship between sleep duration and academic performance, our results suggest an indirect relationship through which students with greater depression, who sleep less, perform worse in school. Finally, we also observed that students who went to bed later and slept for a shorter duration reported higher sleepiness than their earlier peers, an unsurprising but significant correlation. Increased sleepiness did not hinder academic performance in our study; however, the negative consequences of insufficient sleep can extend beyond the classroom. Given the high rates of substance abuse and motor vehicle accidents among sleep deprived college students[9], we would still strongly advise healthier sleep habits for our students.

Limitations

While this study represents the largest collection of actimetry data in undergraduate students across seasons, it was limited to a single institution in the Pacific Northwest. Given that seasonal variations in photoperiod are determined by latitude, it is important that similar

data be collected from students living in latitudes closer to the equator where daylight is more consistent throughout a year. In addition, our population exclusively contained students enrolled in a biology course. This may bias our sample towards students in STEM fields and not represent the behavior of students in the arts or humanities. Furthermore, in quarters where we sampled a subset of students due to resource constraints (see Methods), the pool of potential participants was first narrowed by the professor teaching the course to limit the sample to students who would be reliable participants before soliciting volunteers. This may have biased our sample to higher achieving students and introduced a volunteer bias that could not be accounted for. Finally, we limited our analysis to a subset of the sleep variables which were collected. It is possible that other significant and relevant relationships exist between variables not tested and further work should be done using the unique dataset reported here.

Future Research and Policy Suggestions

The results from this study establish a solid foundation for future work. First and foremost, similar research needs to be done at institutions from a variety of latitudes and time zones. As we have established, latitude determines seasonal changes in photoperiod and thus likely influences sleep and other factors across a year. Similarly, schools on the eastern and western edges of the same time zone at the same latitude will have a different solar noon due to the difference in longitude. Therefore, it is possible that the sleep patterns of students from two such schools are different and require different policy changes to address their needs. Second, it would be interesting to see how sleep patterns and other variables change in the same students over academic years. The mood results reported in this study suggest there may be differences between academic year, but more research needs to be done to address these potential changes. Third, in order to further clarify how sleep affects academic performance it would be ideal to perform a pseudo-controlled field study where undergraduate students are randomly assigned to sleep different amounts and at different times throughout a week.

In this study we established strong evidence for a seasonal rhythm in humans and furthered the understanding of the relationships between sleep, academic performance, and student affect. We also established that undergraduates in our institutions are still not

averaging healthy amounts of sleep each night. To address this problem, we encourage school administrations to take several steps. First, set a limit on the scheduling of morning classes to allow students time to sleep later and increase their nightly duration. Depending on latitude, this limit may need to be adjusted by season to account for changes in the local photoperiod. Second, contemplate changes in lighting within dorms and common spaces that increase morning light exposure and decrease evening light during the winter. Third, limit submission deadlines in the evenings and/or early mornings. Midnight (or later) deadlines incentivize late-night academic work, which may be furthering the achievement gap between early and late chronotype students. Finally, we encourage universities to include information on the importance of sleep in all orientation materials. In our experience, many students are unaware of the ways that nightly sleep can influence their daily lives and thus are making uninformed decisions about personal schedules.

TABLES AND FIGURES

Year	Quarter	Class	Subjects (F)	Dates
2015	Fall	220	102 (62)	10/6 – 11/19
2016	Spring	418	62 (39)	3/30 – 5/16
2016	Summer	220	68 (40)	6/27 – 7/28
2016	Fall	220	36 (32)	10/31 – 11/16
2017	Spring	418	57 (26)	3/24 – 4/26
2017	Summer	220	30 (21)	7/11 – 7/26
2018	Winter	220	88 (57)	1/8 – 2/23
2018	Spring	418	64 (35)	4/4 – 5/8

Table 1 Summary of data collection dates. Data was acquired from students taking either biology 220 or 418 at the University of Washington in two-week intervals. In quarters where the number of participants exceeded the number of available watches, students were divided into cohorts with one cohort participating in data collection followed by a second cohort, until all available subjects participated.

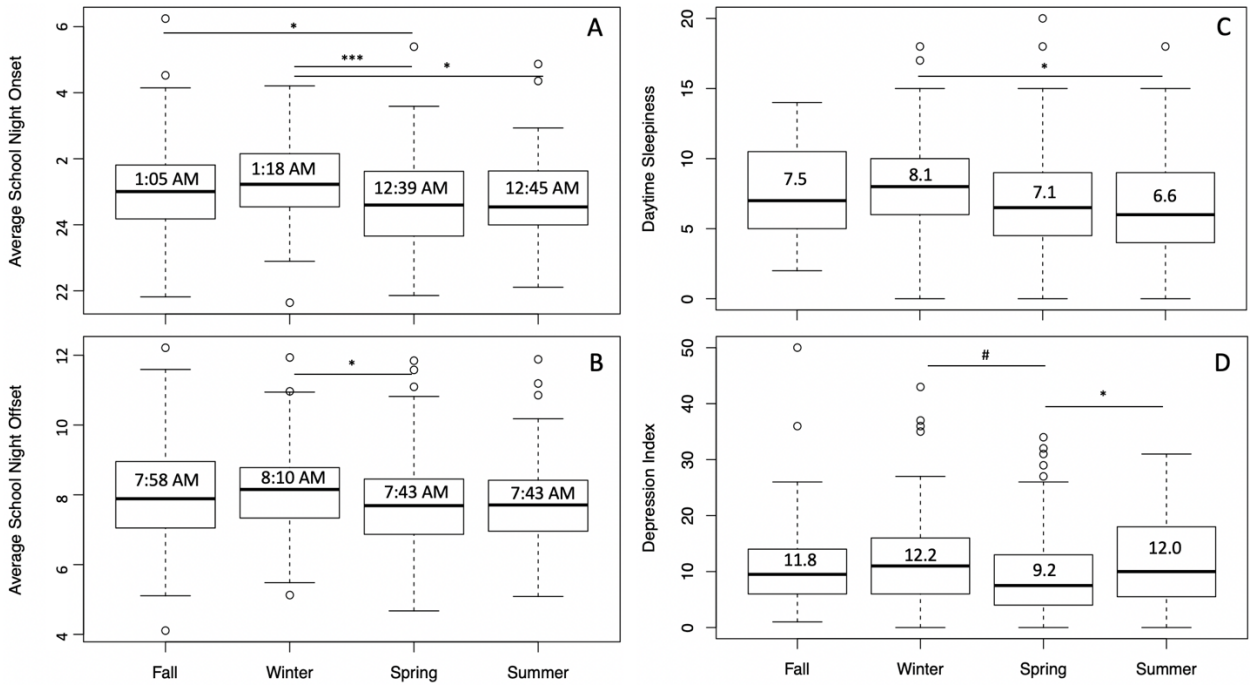


Figure 1. Seasonal variation in sleep, mood, and sleepiness in college students. Box and whisker plots of mean Onset (A) and Offset (B) for School Nights, Sleepiness (C) and Mood (D) by quarter. Means for each observation are represented by the bold black line in the center of the box with the upper and lower quartiles represented by the limits of each box. Means are annotated numerically above each corresponding line. Outliers are represented by individual circles. There was a significant main effect for sleep onset and offset (Kruskal-Wallis X^2, $p < 0.001$ and $p = 0.028$ respectively), sleepiness ($X^2_{(3)} = 8.9441$, $p = 0.030$), and mood (Kruskal-Wallis $X^2_{(3)} = 10.331$, $p = 0.016$). Dunn's comparisons with Bonferroni correction were used for post-hoc tests (#$p < 0.01$, *$p < 0.05$, ***$p < 001$).

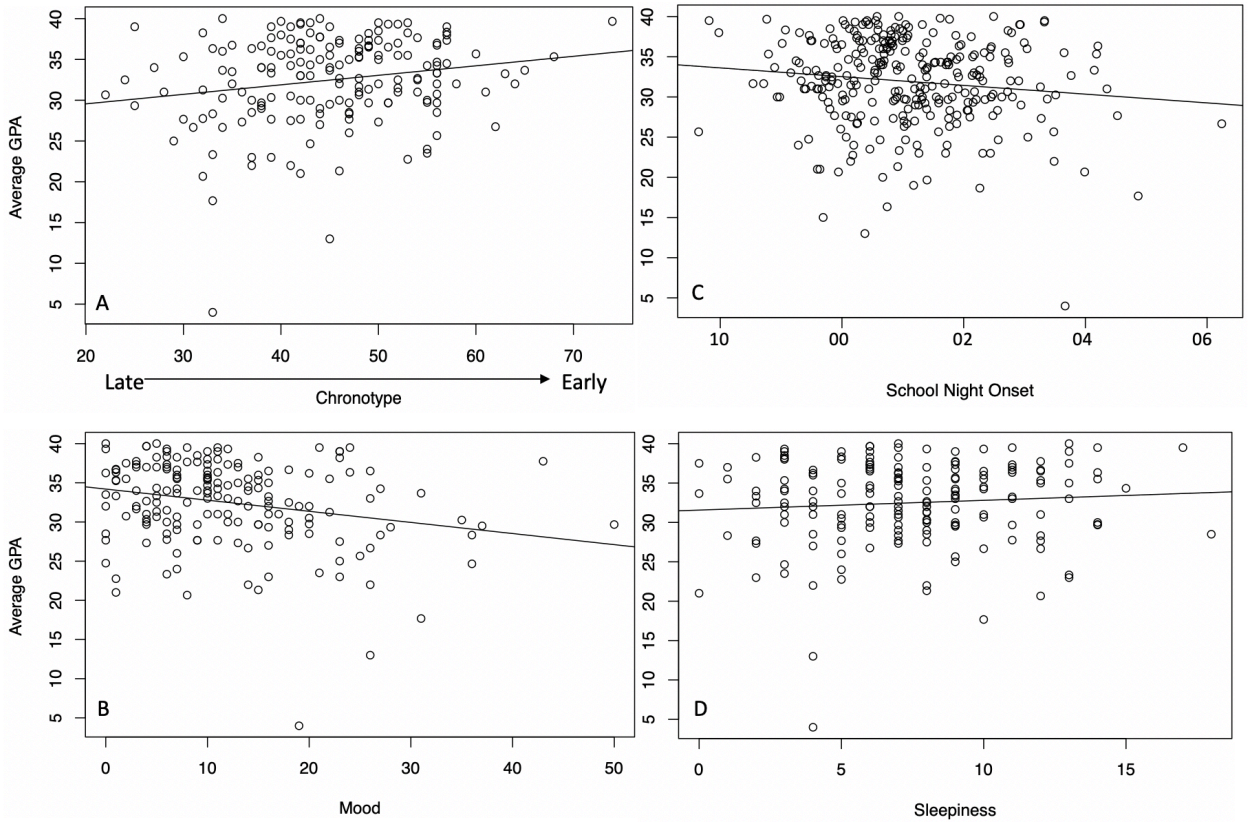
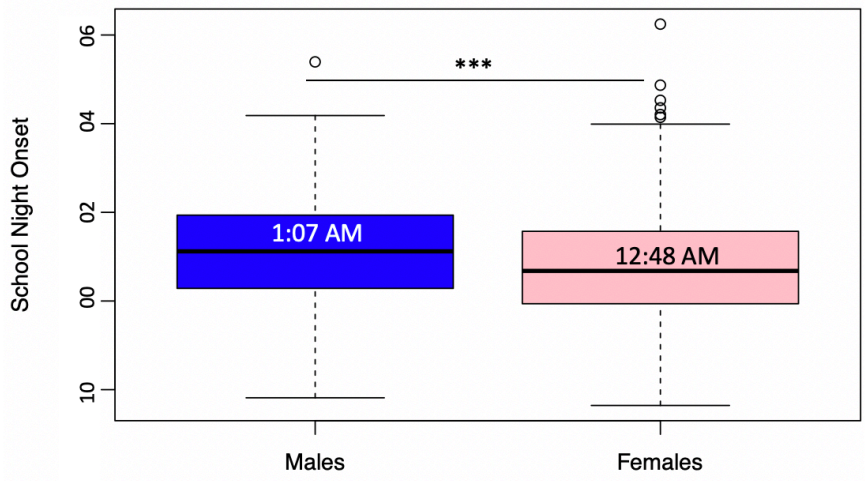


Figure 2. Association between chronotype (A), mood (B), school night onset (C), and sleepiness (D) and academic performance. Average GPA was calculated for each student from the GPA's of each class the student attempted during the quarter they were a study participant. While GPA is measured on a 4.0-scale, the office of the registrar reports GPA on a 40-point scale as represented above. Predictors of academic performance were assessed using a linear model selection paradigm (see Methods). Due to the significant association between Chronotype and Sleep Onset (Sup. Fig. 1), separate models were run with only one of those variables. The first single model of best fit emerged ($F_{(3, 169)} = 7.379$, $p < 0.001$) with Chronotype ($\beta = 0.174$, $p = 0.007$), Mood ($\beta = -0.207$, $p < 0.001$), and Sleepiness ($\beta = 0.129$, $p = 0.041$) as significantly associated with academic success. The second single model of best fit emerged ($F_{(3,169)} = 6.953$, $p < 0.001$) with Average School Night Onset ($\beta = -0.181$, $p = 0.014$), Mood ($\beta = -0.22$, $p < 0.001$) and Sleepiness ($\beta = 0.139$, $p = 0.03$) emerging as significantly associated with academic performance.

A



B

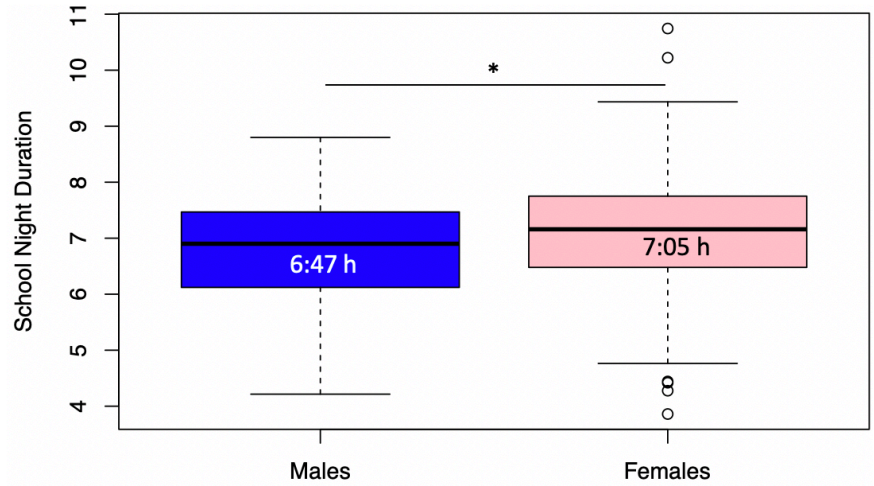


Figure 3. School night sleep differs by sex. Box and whisker plots for Average School Night Onset and Duration. Means for each observation are represented by the bold black line in the center of the box with the upper and lower quartiles represented by the limits of each box. Means are annotated numerically above (A) or below (B) each corresponding line. Outliers are represented by individual circles. Predictors of average School Night Onset and Duration were assessed using a linear model selection paradigm (see Methods). The model of best fit for Onset ($F_{(14, 336)} = 21.61$, $p < 0.0001$) revealed that females went to sleep significantly earlier than males ($p < 0.001$). The model of best fit for Duration ($F_{(11, 338)} = 6.109$, $p < 0.0001$) revealed that females slept significantly longer than males ($p = 0.004$).

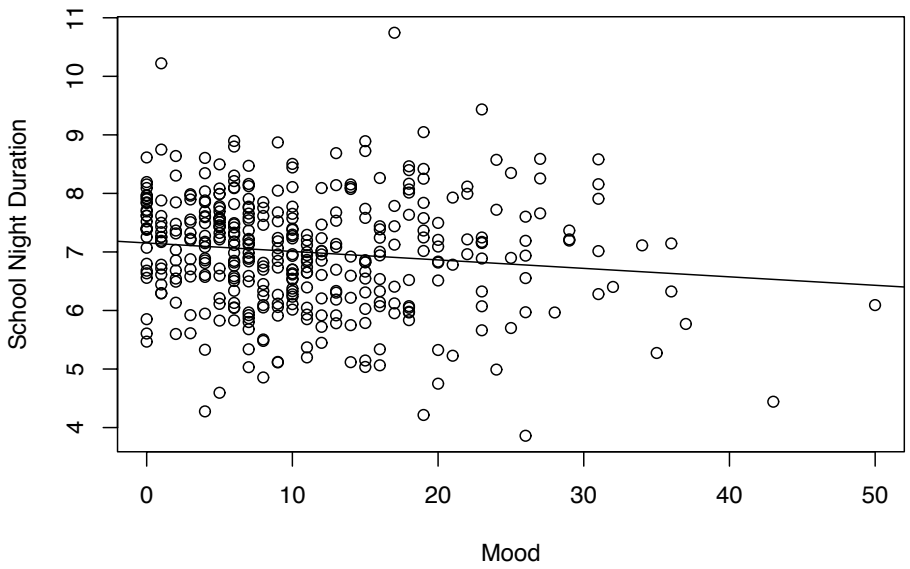
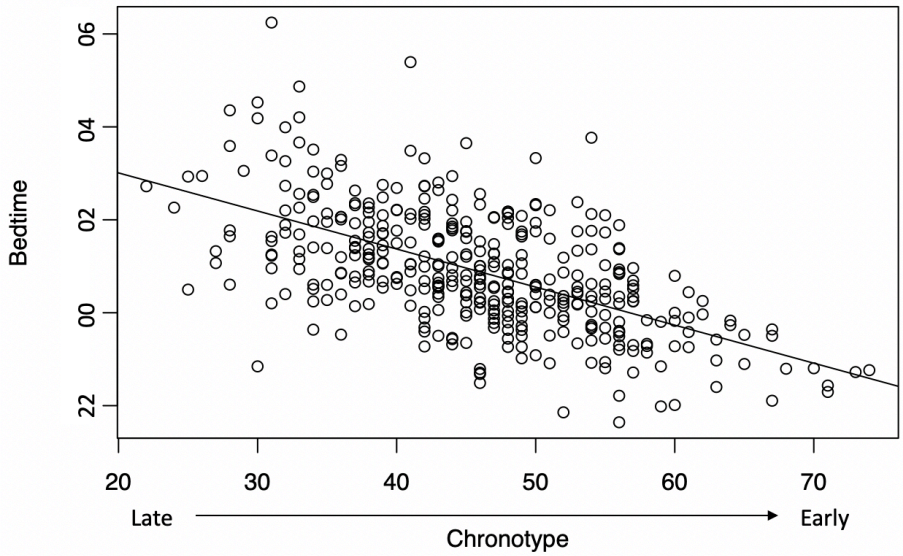


Figure 4. Sleep duration is negatively associated with mood. Higher mood scores represent higher depression. Predictors of average School Night Duration were assessed using a linear model selection paradigm (see Methods). The model of best fit for Duration ($F_{(11, 338)} = 6.109$, $p < 0.0001$) revealed a significant relationship between mood and duration ($\beta = -0.10071$, $p = 0.02$)

Supplementary Figures



Supplementary Figure 1. Association between average school night onset and chronotype as measured by the Horne-Östberg Chronotype Questionnaire. A simple linear model reveals that higher chronotype scores were significantly associated with earlier school night onsets ($F_{(1, 371)} = 126.8$, Adjusted $R^2 = 0.2527$, $p < 0.0001$).

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APPENDIX

Linear Model Selection for Academic Performance

We used a linear model selection paradigm to determine what variables contributed to academic performance in our population. For each model, the outcome of academic performance as measured by the average GPA for all classes across the quarter. To avoid issues of multicollinearity, four different models were fit to the data. Each of the four models began with the same set of explanatory variables (see below) but contained a different demographic variable (Sex, FG, URM, EOP). The full model was:

GPA ~ Quarter + Year + Chronotype + Average Weekday Duration + Social Jetlag + Mood score + Sleepiness score + [Demographic Variable (Sex, FG, URM, EOP)]

For all models, the reference categories were as follows: Sex (males), FG (nonFG), URM (nonURM), EOP (nonEOP). Once a model was run, one of the explanatory variables that did not significantly contribute to the outcome was removed and a second model was run. To assess the impact of the missing variable, a likelihood ratio test (ANOVA) was run and the AIC values of the two models were compared. If there was no significant difference between the models as shown by the results of the ANOVA and if the AIC value for the second model was lower, model selection continued. This process continued until there were no more non-significant variables to remove, or the results of the ANOVA and AIC indicated that removing a variable resulted in a weaker model. Once this process was complete for all four student subpopulations the results were compared. In three of the four cases, a single model of best fit emerged and is summarized below:

GPA ~ Chronotype + Mood score + Sleepiness score

A summary of the regression statistics is provided in Table A

Table A. Regression statistics: predicting GPA

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.71798	0.04326	16.597	< 0.001
horne_type	0.17423	0.06427	2.711	0.007
mood_score	-0.20698	0.06161	-3.360	< 0.001
sleepiness_score	0.12876	0.06247	2.061	0.04

Residual standard error: 0.1437 on 169 degrees of freedom
Multiple R-squared: 0.1158
Adjusted R-squared: 0.1001
F-statistic: 7.379 on 3 and 169 DF
p-value: 0.0001124

In one of the four cases, a different model of best fit emerged and is summarized below:

GPA ~ FG + chronotype + Mood score + Sleepiness score

A summary of the regression statistics is provided in Table B

Table B. Regression statistics: predicting GPA with FG Status

	Estimate	Std. Error	t value.	Pr(>|t|)
(Intercept)	0.72668	0.04323	16.810	< 0.001
FG	-0.05124	0.02809	-1.824	0.07
horne_type	0.16355	0.06410	2.551	0.012
mood_score	-0.19384	0.06161	-3.146.	0.002
sleepiness_score	0.13573	0.06216	2.184	0.03

Residual standard error: 0.1427 on 168 degrees of freedom
Multiple R-squared: 0.133
Adjusted R-squared: 0.1123
F-statistic: 6.442 on 4 and 168 DF
p-value: 7.574e-05

Preliminary analysis indicated that Average School Night Bedtime may have had a significant effect on academic performance. As a result, one additional set of models was selected with Average School Night Onset instead of Average School Night Duration as an explanatory variable. Due to the tight association between Average School Night Onset and chronotype, the results of the Horne-Ostberg questionnaire were not included in the model. A summary of the full model is below:

GPA ~ Quarter + Year + Average Weekday Onset + Social Jetlag + Mood score + Sleepiness score + [Demographic Variable (Sex, FG, URM, EOP)]

In three of the four cases, a single model of best fit emerged and is summarized below:

GPA ~ Average Weekday Onset + Mood score + Sleepiness score

A summary of regression statistics is provided in Table C

Table C. Regression statistics: predicting GPA with Average Weekday Onset

	Estimate	Std. Error.	t value.	Pr(>|t|)
(Intercept)	0.87012	0.03707	23.474	< 0.001
Average.Weekday.Onset	-0.18079	0.07278	-2.484	0.01
mood_score	-0.21982	0.06147.	-3.576.	< 0.001
sleepiness_score	0.13937	0.06359.	2.192	0.03

Residual standard error: 0.1442 on 169 degrees of freedom
Multiple R-squared: 0.1099
Adjusted R-squared: 0.09406
F-statistic: 6.953 on 3 and 169 DF
p-value: 0.0001935

In one of the four cases, a different model of best fit emerged and is summarized below:

GPA ~ FG + Average Weekday Onset + Mood score + Sleepiness score

A summary of the regression statistics is provided in Table D

Table D. Regression statistics: predicting GPA with Average Weekday Onset and FG

	Estimate.	Std. Error	t value	Pr(>|t|)
(Intercept)	0.87900	0.03681	23.879.	< 0.001
FG	-0.06437	0.02801	-2.298	0.02
Average.Weekday.Onset	-0.19521	0.07215.	-2.705	0.007
mood_score	-0.20142	0.06123	-3.289	0.001
sleepiness_score	0.15272	0.06307	2.422	0.017

Residual standard error: 0.1424 on 168 degrees of freedom
Multiple R-squared: 0.137
Adjusted R-squared: 0.1164
F-statistic: 6.667 on 4 and 168 DF
p-value: 5.276e-05

Linear Model Selection for Sleep

To determine what variables may be contributing to differences in student sleep, a similar method of model selection was implemented to the one described above. In this case, two sleep variables were selected as the outcomes: Average School Night Onset and Duration. There was no *a priori* reason to believe that FG, URM, and EOP status may have a significant effect on sleep. Thus, initial models for both sleep outcomes included Quarter, Year, Sex, Ethnicity, Chronotype, and Mood. Ethnicity was selected over Race as an explanatory variable because ethnicity had fewer categories and thus each category contained a larger and more meaningful sample size. The full model for Average Weekday Onset is summarized below:

Average Weekday Onset ~ Quarter + Year + Sex + Ethnicity + Chronotype + Mood score

The full model for Average Weekday Duration is summarized below:

Average Weekday Duration ~ Quarter + Year + Sex + Ethnicity + Chronotype + Mood score

For all models, the reference categories were as follows: Sex (males), Ethnicity (Caucasian). For both sleep outcomes, model selection proceeded in the exact same manner as above using backward selection while comparing the ANOVA and AIC values for each subsequent model until a best fit was determined. To predict Average Weekday Onset, the model of best fit is summarized below:

Average Weekday Onset ~ Quarter + Sex + Ethnicity + Chronotype

A summary of regression statistics is provided in Table E

Table E. Predicting Average Weekday Onset

	Estimate	Std. Error	t value.	Pr(>|t|)
(Intercept)	0.6890396	0.0293970	23.439	< 0.001
QuarterSpring	-0.1294606	0.0234318	-5.525	< 0.001
QuarterSummer	-0.1189827	0.0257631	-4.618	< 0.001
QuarterWinter	-0.0758371	0.0255987	-2.963	0.003
Sex	-0.0163362	0.0447316	-0.365	0.72
SexF	-0.0408144	0.0127888	-3.191	0.002
Ethnicity	0.0004686	0.0282075	0.017	0.99

EthnicityAFRO-AM	0.0307728	0.0675939	0.455	0.65
EthnicityASIAN	0.0544441	0.0156050	3.489	< 0.001
EthnicityHAW/PAC	-0.0856473	0.0817794	-1.047	0.30
EthnicityHISPANIC	0.0312146	0.0246565	1.266	0.21
EthnicityINTERNATIONAL	0.1237120	0.0255348	4.845	< 0.001
EthnicityMULTI	0.0390724	0.0193434	2.020	0.05
EthnicityNOTIND	0.0571532	0.0456591	1.252	0.21
horne_type	-0.4566539	0.0331724	-13.766	< 0.001

Residual standard error: 0.1146 on 367 degrees of freedom
Multiple R-squared: 0.4653
Adjusted R-squared: 0.4449
F-statistic: 22.81 on 14 and 367 DF
p-value: < 2.2e-16

Due to low sample sizes, the significant relationship between students who identify as "MULTI" for ethnicity and Average Weekday Onset was not investigated further. To predict Average Weekday Duration, the model of best fit is summarized below:

Average Weekday Duration ~ Sex + Ethnicity + Mood score

A summary of regression statistics is provided in Table F

Table F. Predicting Average Weekday Duration

	Estimate.	Std. Error	t value	Pr(>|t|)
(Intercept)	0.4924472	0.0163632	30.095	< 0.001
Sex	-0.0197231	0.0523476	-0.377	0.71
SexF	0.0417806	0.0146494	2.852	0.005
Ethnicity	-0.0227963	0.0306617	-0.743	0.46
EthnicityAFRO-AM	-0.1936485	0.0779254.	-2.485	0.013
EthnicityASIAN	-0.0981942	0.0175168	-5.606	< 0.001
EthnicityHAW/PAC	0.0007243	0.0950179	0.008	0.99
EthnicityHISPANIC	-0.0259510	0.0285984	-0.907	0.36477
EthnicityINTERNATIONAL.	-0.0217068	0.0294613	-0.737	0.46172
EthnicityMULTI	-0.0375098	0.0224534	-1.671	0.096
EthnicityNOTIND	-0.0999183	0.0517707	-1.930	0.05
mood_score	-0.1112649	0.0411931	-2.701	0.007

Residual standard error: 0.1334 on 369 degrees of freedom
Multiple R-squared: 0.1288
Adjusted R-squared: 0.1028

F-statistic: 4.957 on 11 and 369 DF
p-value: 3.4e-07

Due to low sample sizes, the significant relationship between students who did not provide identification for ethnicity and Average Weekday Duration was not investigated further.

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