


BMJ Open The CLASS Study (Circadian Light in Adolescence, Sleep and School): protocol for a prospective, longitudinal cohort to assess sleep, light, circadian timing and academic performance in adolescence

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ABSTRACT

Background During adolescence, sleep and circadian timing shift later, contributing to restricted sleep duration and irregular sleep-wake patterns. The association of these developmental changes in sleep and circadian timing with cognitive functioning, and consequently academic outcomes, has not been examined prospectively. The role of ambient light exposure in these developmental changes is also not well understood. Here, we describe the protocol for the Circadian Light in Adolescence, Sleep and School (CLASS) Study that will use a longitudinal design to examine the associations of sleep-wake timing, circadian timing and light exposure with academic performance and sleepiness during a critical stage of development. We also describe protocol adaptations to enable remote data collection when required during the COVID-19 pandemic. **Methods** Approximately 220 healthy adolescents aged 12–13 years (school Year 7) will be recruited from the general community in Melbourne, Australia. Participants will be monitored at five 6 monthly time points over 2 years. Sleep and light exposure will be assessed for 2 weeks during the school term, every 6 months, along with self-report questionnaires of daytime sleepiness. Circadian phase will be measured via dim light melatonin onset once each year. Academic performance will be measured via national standardised testing (National Assessment Program—Literacy and Numeracy) and the Wechsler Individual Achievement Test—Australian and New Zealand Standardised Third Edition in school Years 7 and 9. Secondary outcomes, including symptoms of depression, anxiety and sleep disorders, will be measured via questionnaires.

Discussion The CLASS Study will enable a comprehensive longitudinal assessment of changes in sleep-wake timing, circadian phase, light exposure and academic performance across a key developmental stage in adolescence. Findings may inform policies and intervention strategies for secondary school-aged adolescents.

Ethics and dissemination Ethical approval was obtained by the Monash University Human Research Ethics

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study will use a longitudinal design to comprehensively and prospectively examine the relative contributions of multiple sleep and circadian factors to academic performance and sleepiness, for 2 years during adolescence (age 12–13 to 14–15 years).
- ⇒ A multi-method approach will be used, including objective measurement of sleep timing via wrist actigraphy, gold-standard circadian phase assessments via salivary dim light melatonin onset, and assessment of light exposure using a sensor validated for measurement of melanopic illuminance.
- ⇒ Results from this study may inform future development of practical tools to minimise sleep-wake disturbances in healthy adolescents, such as sleep-wake and light monitoring systems.
- ⇒ This is a naturalistic observational study; although key confounders are measured (e.g., pubertal status, academic motivation), they are not exhaustive.
- ⇒ Protocol amendments have been made to enable entirely remote data collection where required during the COVID-19 pandemic.

Committee and the Victorian Department of Education. Dissemination plans include scientific publications, scientific conferences, via stakeholders including schools and media.

Study dates Recruitment occurred between October 2019 and September 2021, data collection from 2019 to 2023.

INTRODUCTION

Adolescents worldwide get insufficient sleep, particularly on school nights.^{1–3} Sleep of adequate duration and quality, and at appropriate circadian times, is crucial for physical and mental health and learning.^{4–8}

Experimental and cross-sectional studies show that insufficient and mistimed sleep is associated with reduced cognitive function,⁹ negative mood and poor mental health in adolescents.^{10 11} Adolescence is a critical phase for the development of core cognitive skills, making the high prevalence of insufficient and mistimed sleep a key health concern. How developmental changes in sleep and circadian timing are related to cognitive function and academic achievement is not currently well understood.

A key feature of sleep through adolescence is a progressive delay in bed and wake times,¹² which is most pronounced on weekends and during vacation, when sleep schedules are less prescribed.¹³ Sleep before non-school days is often 2 or more hours later than on school nights,¹ with longer total sleep time.¹⁴ The delay in sleep timing during adolescence is caused by progressive delays in circadian timing and a slowing in the build-up of homeostatic sleep pressure.¹⁵ These developmental processes interact with early school start times, leading to these large differences in sleep-wake timing as adolescents alternate between school and non-school days.^{1 14 16 17} Delay in sleep and circadian timing begins in early to mid-adolescence, with greatest discordance between sleep on weekdays and weekends recorded in mid-adolescence.^{16 17}

Among adolescents, developmental changes in circadian and sleep characteristics are associated with increased daytime sleepiness (reported by nearly 50% of adolescents aged ~14 years), as well as reduced alertness and sustained attention.¹⁸ Short sleep, poor quality sleep and irregular sleep-wake patterns typical of adolescents during school term are also associated with daytime sleepiness,^{19 20} impairments in cognitive functioning^{21 22} and poorer academic performance.^{23 24} A systematic review and meta-analysis showed that shorter sleep duration, poorer sleep quality, and greater sleepiness are each associated with poorer school performance, with sleepiness showing the strongest effect size among the three.⁷ Most prior studies have been cross-sectional and measured sleep and/or academic performance subjectively. Longitudinal studies with objective measures are needed to quantify developmental changes in sleep and circadian timing, and to examine their prospective associations with adolescents' cognitive functions and academic performance.

Light is the strongest time cue that resets the human circadian clock,²⁵ which is especially sensitive to short wavelength (blue) light.²⁶ Light exposure during the evening and early night, even at levels typically experienced in indoor settings, delays the circadian clock^{27 28} and suppresses the synthesis of melatonin.^{29 30} Light is also a stimulant, increasing alertness directly,^{31–33} with responses persisting for several hours after lights are turned off, leading to longer sleep latencies and reduced slow-wave sleep.^{34 35} These effects may be particularly pronounced during adolescence, as circadian sensitivity (measured via melatonin suppression) to evening light exposure is increased during early-mid puberty compared with late puberty.³⁶

Widespread use of technology and increased exposure to blue-enriched light at night may exacerbate the tendency to delay circadian timing and delay bedtime in adolescents.³⁷ Use of electronic devices (including those that emit bright and blue-enriched light and are held close to the eyes) is widespread among adolescents: (i) 82% of children in Australia aged 12–13 years regularly using a smartphone in 2020³⁸ and (ii) 71.5% of adolescents reported using at least one light-emitting electronic device in the hour before bed, which is associated with insufficient sleep duration on school nights.³⁹ Detailed longitudinal assessments of light exposure during adolescence and the relationship with sleep and circadian timing, have not yet been conducted.

Current study

Although many studies have examined sleep-wake timing among adolescents of different ages, there are few longitudinal studies, and none examining the effects of objectively measured sleep and circadian timing on academic outcomes. This prospective, longitudinal study aims to comprehensively examine the relationships among sleep and circadian timing, cognitive, academic, and sleepiness outcomes, while accounting for pubertal status, environmental light exposure, and social constraints (e.g., school start times, extracurricular activities). Specifically, the primary aims of the Circadian Light in Adolescence, Sleep and School (CLASS) study are as follows:

1. To quantify longitudinal changes in sleep metrics (e.g., timing, duration, quality and/or regularity), circadian timing (measured by salivary dim light melatonin onset (DLMO)), and circadian timing of sleep (i.e., time between DLMO and sleep on/offset times) in adolescence using both objective and self-report measurements and to explore predictors of trajectories of these metrics;
2. To quantify how sleep metrics and circadian timing are predicted by environmental light exposure patterns using an established biomathematical model of the human circadian system^{37 40};
3. To quantify how changes in subjective sleepiness over time are predicted by changes in sleep metrics, circadian timing, and circadian timing of sleep; and
4. To quantify how indicators of academic performance over time are predicted by sleep metrics, circadian timing, and circadian timing of sleep, with subjective sleepiness acting as a mediator of these effects.

In secondary analyses, we will examine (i) longitudinal relationships between sleep metrics and circadian timing, light exposure and mood; and (ii) interrelationships between social and behavioural factors and sleep-wake outcomes.

METHODS

Design

The study will use a prospective, longitudinal measurement burst design, with five waves of data collection

at 6-month intervals (T0 baseline; T1 6 months; T2 12 months; T3 18 months; T4 24 months), where participants are aged ~12–13 years at T0 and ~14–15 years at T4. The longitudinal design will enable examination of potential causal mechanisms associated with sleep, circadian timing (melatonin rhythm), light exposure, and academic performance, which is not possible with cross-sectional designs. The baseline participant age of ~12 years was selected as an age prior to the expected onset of adolescent sleep and circadian changes,¹⁶ thus maximising the opportunity to study the development and temporal associations of sleep, circadian timing, light exposure and academic performance.

Data will be collected during school term, at least 1-2 weeks after the end of vacations to control for transition effects. During each wave, participants will complete 2 weeks of daily at-home sleep and light monitoring, and psychosocial assessments. Two weeks was selected to enable reliable calculation of the Sleep Regularity Index (SRI)⁴¹, and to capture 4 weekend days to allow meaningful weekday vs weekend (i.e., school vs non-school) comparisons.

Participants and recruitment

Participants will be 220 Year 7 students (approximately equal numbers of males and females) meeting the following inclusion criteria: aged 12–13 years at study entry, attending secondary school in Melbourne, VIC, Australia. Participants will be required to have daily access to a smartphone to complete daily surveys, and be intending to sit the National Assessment Program-Literacy and Numeracy test (NAPLAN) in Year 7 and Year 9. Exclusion criteria include current parent reported diagnosis by a medical professional of (i) a sleep disorder other than Delayed Sleep Wake Phase Disorder (DSWPD) or insomnia, (ii) severe psychiatric (depressive, bipolar, schizophrenia/psychotic, substance related, obsessive-compulsive, feeding and eating, and trauma-related and stressor-related disorders) or (iii) neurodevelopmental disorders (autism spectrum disorder, attention-deficit/hyperactivity disorder, intellectual disabilities or cognitive disorders such as traumatic brain injury). These exclusion criteria were selected due to the potential to confound our primary outcome measures of sleep, circadian timing and academic performance.

Recruitment strategy

Participants will be recruited via advertisements online, in local community spaces in the Melbourne area, and via local secondary schools. Recruitment will target a sample representative of the population on gender, ethnicity, school type and socioeconomic status from the schools in the Melbourne/Monash school catchment area. Demographics and socioeconomic status will be collected to examine how final sample characteristics compare with those Australia-wide.

Sample maintenance/retention

To reduce missing data, efforts will be made to maintain regular contact with participants, including a birthday

card and movie voucher each year, as well as annual holiday greeting cards and newsletters. Every 6 months, parents will be asked to update contact details, school attendance and medical information about the participating adolescents. Participants and their parents will be reimbursed up to a total of \$280 for their time and effort throughout the study. Reimbursements will be made periodically based on task completion, including bonuses when completing all tasks, in an effort to improve retention. Given the longitudinal nature of the study, the monetary value may change if additional measures are added in the future.

Estimation of statistical power

No previous studies have included longitudinal sleep and circadian assessments with standardised academic tests in adolescents. Cross-sectional literature for individual constructs shows generally small to moderate effects. Given that very small effects are not of substantial practical significance, power analyses were designed to detect standardised regression coefficients of 0.25–0.35. Based on 1000 Monte Carlo simulations conducted in MPlus, and assuming a 10% drop-out rate,⁴² the proposed sample size of approximately 220 will provide >80% power for direct and mediated effects in Aims 2–3.

Patient and public involvement

A patient and public involvement (PPI) group of relevant stakeholders (e.g., parents, teachers) will be established to meet regularly with the research team. The PPI group will advise on recruitment and research procedures, interpretation of study results and dissemination, and discussion of future interventions.

Procedures

Figure 1 summarises the timing of study procedures.

Screening and consent

Potential participants will be sent a medical screening survey to assess eligibility, completed by the parent. Eligible participants will be sent detailed study information and an explanatory video. A telephone call will then be scheduled to confirm eligibility and answer questions prior to the participant and their parent both providing written informed assent and consent.

Contact and timing of assessments

Prior to each wave, the parent will complete a medical update questionnaire, covering any new medical diagnoses, medications, changes in contact details or school, or recent travel across time-zones. Travel across time-zones within the last month will result in rescheduling data collection until at least 1 month after the participant has returned to Melbourne, Australia local time (Australian Eastern Time). Medications that may influence sleep or melatonin production/synthesis (e.g., beta-blockers or SSRIs) will be recorded and accounted for during analysis. New medical diagnoses, including psychiatric diagnoses, will not be considered exclusionary, unless they prevent

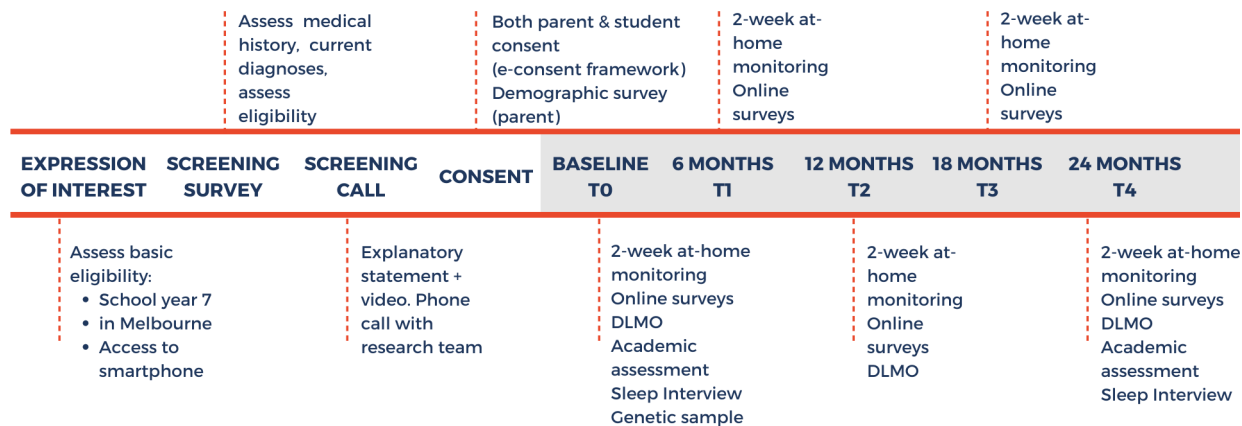


Figure 1 Protocol flow chart for the Circadian Light in Adolescence, Sleep and School (CLASS) Study. Data are collected in five waves, each approximately 6 months apart, starting when the participant is in Year 7 (age 12–13 years). Academic assessments and sleep interviews occur within ± 2 weeks of the Dim Light Melatonin Onset (DLMO) assessment.

the participant from comfortably and safely completing the study measures.

Six-monthly assessments

During each wave of data collection (T0, T1, T2, T3, T4; 6-month intervals ± 2 months), participants will complete 2 weeks of at-home sleep and light monitoring (see figure 1). To measure sleep, participants will wear a wrist-worn actigraphy device (GENEActiv Original, Activinsights, UK). To measure daily light exposure, participants will wear a light pendant (ActTrust, model code AT0503LF, Condor Instruments, Brazil). Daily surveys will be administered via a mobile phone app (Metricwire) installed on the participant's phone. Surveys will be available at fixed times, and include a sleep and screen use diary asking about sleep the night before (open between 6:00 and 23:59), a morning sleepiness survey (open between 6:00 and 11:00), and an afternoon affect and sleepiness survey (open between 15:00 and 19:00). During the 2-week monitoring, participants will also complete a series of online questionnaires (see table 1), administered via REDCap (Research Electronic Data Capture^{43 44}).

Videos with demonstrations of study procedures will be sent to participants at each wave to aid adherence. Email reminders will be used to encourage survey completion, and push notifications and text message reminders will be generated by Metricwire each day to improve compliance with daily measures. The research team will monitor survey completion and follow-up with any participants who may need assistance.

Yearly assessments

At annual data collection waves (T0, T2, T4; 12-month intervals ± 2 months), participants will complete a circadian phase assessment on the final Friday of the at-home monitoring. Participants will conduct this either at the Turner Institute for Brain and Mental Health (Monash University), or in their home when required during the COVID-19 pandemic. In both locations, saliva samples will be collected hourly, from 4 hours before (to minimise

impact on school attendance), until 2 hours after average sleep onset of the prior week, calculated using self-reported sleep timing measured during the at-home monitoring. The earliest arrival time will be set to 16:00 h, to allow sufficient travel time from school. Participants will be asked to refrain from eating foods high in tryptophan or eating or drinking caffeine in the 24 hours prior to the assessment. Participants will remain seated, with no food or drink intake for at least 20 minutes before each sample.

Laboratory-based collections will take place in a light-controlled auditorium with no windows, where the only light source is a projector screen on 50% brightness, with a blue light filter (f.lux, 1900K setting; <https://justgetflux.com>). Light intensity at eye level of the participants will be confirmed to be < 3 lux, using a light metre (J17 Luma Colour, Oregon), with spectral characteristics recorded using an MK350N spectrometer (UPRTEK, Zhunan, Taiwan). Participants will be provided with light-filtered goggles (209 Neutral Density Filter, LEE, Hampshire, UK; measured incoming light < 1 lux in room lighting of 500 lux) when going to the bathroom. Samples will be stored at -20°C prior to assay. These assessments will be conducted in groups of up to 30 participants, based on procedures previously tested in young adults.⁴⁵

For in-home collection, a saliva sampling kit will be posted to participants, along with plastic welder's goggles and a small handheld torch, both fit with neutral density filters described above (see online supplemental figure S1). Participants will be asked to prepare a room in their home that can remain dark (goal < 3 lux) throughout the assessment. During the assessment, participants will be monitored via group video conferencing, with research team members present to ensure compliance, monitor light levels (confirmed retrospectively using the light pendant data), and to provide guidance throughout the collections. Based on our internal testing, we found that electronic devices (phone, laptop) must be at least

Table 1 Measures and the time(s) of collection planned for the CLASS Study

Primary/ Secondary Outcome	Measures	Frequency	T0	T1	T2	T3	T4
			0 mths	6 mths	12 mths	18 mths	24 mths
Primary	National Assessment Program Literacy and Numeracy (NAPLAN)	Two-yearly	✓				✓
	WIAT-III A&NZ subtests: numerical operations & reading comprehension	Year 7 & Year 9	✓				✓
	Dim light melatonin onset (DLMO)	Annual	✓		✓		✓
	Objective Sleep Timing, Duration, and Regularity - Wrist accelerometer (GENEActiv Original) and Sleep Diary (daily)	6-Monthly	✓	✓	✓	✓	✓
	Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD)		✓	✓	✓	✓	✓
	Light exposure (ActTrust device with specialized filter, worn around the neck)		✓	✓	✓	✓	✓
Covariate	Pubertal Development Scale	6-Monthly	✓	✓	✓	✓	✓
	Test Anxiety Inventory		✓	✓	✓	✓	✓
	Academic Motivation Scale		✓	✓	✓	✓	✓
	Strengths and Difficulties Questionnaire		✓	✓	✓	✓	✓
	Structured Clinical Interview for Sleep Disorders (SCISD)	Two-yearly	✓				✓
Secondary	Karolinska Sleepiness Scale (daily: morning & afternoon)	6-Monthly	✓	✓	✓	✓	✓
	Positive and Negative Affect Schedule-X (daily: afternoon)		✓	✓	✓	✓	✓
	Screen use in 1h before bed (daily)		✓	✓	✓	✓	✓
	Perceived Stress Scale		✓	✓	✓	✓	✓
	Depressive Symptoms (PROMIS Pediatric CAT v2.0)		✓	✓	✓	✓	✓
	Anxiety (PROMIS Pediatric CAT v2.0)		✓	✓	✓	✓	✓
	Peer Relationships (PROMIS Pediatric CAT v2.0)		✓	✓	✓	✓	✓
	Family Relationships (PROMIS Pediatric CAT v1.0)		✓	✓	✓	✓	✓
	Cognitive Function (PROMIS Pediatric CAT v1.0)		✓	✓	✓	✓	✓
	Fatigue (PROMIS Pediatric CAT v1.0)		✓	✓	✓	✓	✓
	Sleep Disturbance (PROMIS Pediatric CAT v1.0)		✓	✓	✓	✓	✓
	School Sleep Habits Survey		✓	✓	✓	✓	✓
	Delayed Sleep-Wake Phase Disorder Screening Scale		✓	✓	✓	✓	✓
	Dysfunctional Beliefs About Sleep Questionnaire		✓	✓	✓	✓	✓
Demographics	Socio-economic status, age, sex, medical history, school type - Questionnaire (parents)	Baseline only	✓				
Exploratory	Saliva sample for genetic analysis		✓				

CLASS, Circadian Light in Adolescence, Sleep and School; PROMIS, Patient-Reported Outcomes Information System; WIAT-III A&NZ, Wechsler Individual Achievement Test-Australian and New Zealand Standardised third Edition.

one metre away from the participant in a dark room (curtains and doors closed, lights off) to maintain photopic illuminance at eye-level of <3 lux. Therefore, devices in the room used for videoconferencing will have a blue light filter app installed, with brightness set to the lowest setting, and positioned at least one metre from the participant. Samples will be stored in the participant's home freezer overnight and transported the following day to be stored in the laboratory at -20°C .

Two-yearly assessments

At baseline (T0) and 24 months (T4; ± 2 months), participants will also complete an academic assessment and Structured Clinical Interview for Sleep Disorders-Revised (SCISD-R; Taylor *et al.*).⁴⁶ Assessments will be scheduled to

occur after school, between $\sim 16:00$ and $19:00$, within ± 2 weeks of the DLMO assessment. Assessments will be administered by registered provisional/general psychologists under supervision. Academic performance will be measured using two subtests of the Wechsler Individual Achievement Test-Australian and New Zealand Standardised third Edition (WIAT-III): (1) Reading Comprehension and (2) Numerical Operations, administered via the iPad testing interface on Q-Interactive (Pearson Australia Group Pty Ltd). Where in-person assessments are not possible during the COVID-19 pandemic, a remote academic assessment will be conducted by provisional/registered psychologists, via secure video conferencing software and in accordance with Pearson guidelines for remote assessments. Results from the

NAPLAN standardised tests in Year 7 and Year 9 will be obtained via the Victorian Curriculum and Assessment Authority database. Participants will also collect a single saliva sample using the Oragene-DNA sampling kits (OG-500, DNA Genotek, Canada) for genetic analysis.

In cases where participant responses on the SCISD-R are indicative of a probable sleep disorder, or responses on the Patient-Reported Outcomes Information System (PROMIS) Paediatric depressive or anxiety symptoms scales are indicative of depression or anxiety risk, the participant and his/her parent will be sent information about available support services.

Measures

A summary of the measures, and the time points at which they are collected, is presented in [table 1](#).

Demographics and socioeconomic status

Sociodemographic factors of participants and their parent(s) will be assessed at baseline by parent report. Factors assessed will include participant date of birth, year in school, type and location of school, participant sex and gender, race/ethnicity and ancestry, family structure, parental education, parental employment status, household annual income and post code linked to census data for area socioeconomic status.

Primary outcomes

Academic performance

The primary study outcome is academic performance, measured via two methods: (1) the Numerical Operations and the Reading and Comprehension subtests of the WIAT-III⁴⁷ and (2) the NAPLAN standardised test administered nationally in Australia, where available (NAPLAN assessments were cancelled in 2020 due to the COVID-19 pandemic, and resumed in 2021). The WIAT-III subtests are included as an additional validated measure of individual academic achievement. Raw item scores are converted to standardised scores, with a mean of 100 and SD of 15 for the purposes of comparisons between the performance of participants via age-based and/or year-based norms.^{47 48} NAPLAN scores are sensitive to change in both longitudinal and interventional studies.^{49 50}

Sleep

A wrist-worn actigraphy device (GENEActiv Original, Activinsights; sampling frequency 30 Hz) will be used to measure sleep-wake activity. Actigraphy is a well-validated objective measure of sleep-wake timing in adolescents,^{51 52} and the GENEActiv device has been validated against sleep diary and polysomnography data.⁵³

A daily sleep diary will ask questions about sleep the night before, including sleep timing, time taken to fall asleep, sleepiness at bedtime, awakenings during the night, naps, screen use before bed and alarm use. The diary will be used to inform sleep intervals during actigraphy analysis (see below).

Daytime sleepiness

Subjective daytime sleepiness will be measured at each time point using the Epworth Sleepiness Scale-Child and Adolescent version,⁵⁴ which is an adapted version of the adult ESS⁵⁵ validated for adolescents aged 12–18 years. Daily subjective sleepiness will be measured using the Karolinska Sleepiness Scale⁵⁶ in the morning and evening each day.

Light

Daily light exposure will be monitored using a specialised modified light pendant (ActTrust, model code AT0503LF, Condor Instruments, Brazil). The ActTrust device reports red, green, blue and infrared light spectra, and is set to record in 1 min epochs. The modified light sensor is fit with a band-pass filter (535BP210; Omega Optical, Brattleboro, Vermont, USA), to increase spectral alignment of the blue wavelength sensor with the melanopic spectral response curve.⁵⁷ While the GENEActiv Original records light exposure, the sensor is less reliable in low light levels.^{58 59} Therefore, we chose to use the custom ActTrust device, worn as a pendant around the neck as our primary light measure, as it is considered the most suitable commercially available sensor for measuring melanopic irradiance,⁵⁷ which is the most physiologically relevant light measure for circadian research.⁶⁰ The device will be worn around the neck or pinned to a collar during waking hours, and placed sensor-side up on a bedside table during sleep.

Circadian phase

Circadian phase will be measured using salivary DLMO. Melatonin secretion is the most reliable measure of circadian timing in humans, with DLMO widely considered the gold-standard method for measuring circadian phase.⁶¹ Samples will be assayed for melatonin concentration via radioimmunoassay, using procedures developed at the University of Adelaide⁶² and reagents provided by Buhlmann Laboratories (Allschwil, Switzerland). The limit of detection of the assay is 1 pg/mL. DLMO time will be calculated by linear interpolation across time points before and after salivary melatonin concentration increases to, and remains above, a fixed threshold of 4 pg/mL.⁶³ The phase relationship between DLMO and sleep timing will be calculated as the time between DLMO time and average sleep-onset time and/or wake time.

Covariates

The following measures will be considered as covariates for the primary aims of the study, measured at each 6-monthly data collection wave: the Pubertal Development Scale,⁶⁴ the Strengths and Difficulties Questionnaire,^{65 66} the Test Anxiety Inventory⁶⁷ and Academic Motivation Scale.⁶⁸ Given that data will be collected in groups of participants starting at different points throughout the school year, season, school mode (in-person or remote learning), data collection mode (in-laboratory DLMO or

at-home DLMO), and school term will also be considered as covariates.

To control for sleep disorders that may emerge during the study, the SCISD-R⁴⁶ will be used to assess criteria for sleep disorders in the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5).⁶⁹ The SCISD-R is a semi-structured clinician-administered interview that will be used to assess: (i) the presence of current or recent sleep disorders; (ii) past lifetime sleep disorder episodes since the age of 5 years; (iii) changes in possible sleep disorders over the 2-year study period; and (iv) validity of circadian rhythm disorder diagnoses alongside data obtained from diaries and objective circadian phase assessments. We adapted some items to improve relevance for adolescents attending school (e.g., asking about sleep on both school and non-school days for insomnia and delayed sleep phase disorder symptoms), and for applicability for a longitudinal study.

Secondary outcomes

The following secondary outcomes will examine changes in self-reported mood, sleep, social behaviours, and cognition. Questionnaires were selected based on strong psychometric properties and validity for use in adolescents. Outcomes measured using the PROMIS Paediatric scales will include: anxiety and depression symptoms,⁷⁰ peer relationships,⁷¹ family relationships,⁷² sleep disturbances,^{73 74} fatigue⁷⁵ and cognitive function,^{76 77} all administered using well-validated computer adaptive testing.^{78 79} Stress will be measured using the Perceived Stress Scale (10-item version),⁸⁰ and a subset of the Positive and Negative Affect Schedule-X⁸¹ will be assessed daily as measures of state sleepiness and affect. Sleep behaviours and attitudes will be measured using the Pre-Sleep Arousal Scale,⁸² the Dysfunctional Beliefs and Attitudes about Sleep-16,⁸³ the Delayed Sleep-Wake Phase Disorder Screening Questionnaire⁸⁴ and the School Sleep Habits Survey,¹⁷ with minor modifications for the Australian school context (e.g., added train or tram options to public transport options for the travel to school question).

A salivary DNA sample will be collected for subsequent analysis for genes related to sleep and circadian regulation. Circadian rhythm regulation is linked to a number of core clock genes, including from the Period (PER) family, CLOCK and BMAL1.⁸⁵ Variations in clock genes have been related to variations in sleep-wake homeostasis and circadian timing.⁸⁶ For example, PER3 polymorphisms have been linked to DSWPD.^{87 88} Candidate genes will be examined, relative to sleep and circadian outcomes. Due to the limited sample size, this analysis will be exploratory.

Data analysis

Prior to primary analyses, we will produce a descriptive profile of the sample, characterise dropout and missing data, and determine if there is clustering by school. If there is substantial clustering by schools, models will use cluster robust standard errors.

Sleep parameters will be calculated using the R package GENEActiv and GENE Data (GGIR).⁸⁹ GGIR detects sleep using an algorithm⁵³ that looks for sustained inactivity bouts within the main sleep period, which can be guided by a sleep diary. When sleep diary data are not available, the sleep period window is detected using the HDCZA algorithm.⁹⁰ Where there are discrepancies of >30 min between GGIR-calculated sleep and the sleep diary, two independent researchers will visually inspect activity and light data, and modify the sleep interval. If the two researchers do not agree, a third researcher will review the data to assist in a final consensus decision. Using these sleep data, we will measure average sleep duration and derive two sleep regularity measures: the SRI²³, and the difference in sleep midpoint between school days and non-school days.⁹¹ Growth scale scores will be calculated for the WIAT-III subscales to measure academic progress over time within participants.⁴⁸ Pubertal scores will be classified into three stages of development (pre-early, mid-pubertal and late/postpubertal).⁹²

For Aim 1, we will use latent growth models to explore longitudinal change trajectories in sleep metrics and circadian timing. We will also explore how different factors (e.g., sex, pubertal status) may predict change trajectories by including them as predictors of intercepts and slopes. We will evaluate model fit to determine whether the change is linear and include non-linear change if it significantly improves fit.

For Aim 2, we will use 24 hour minute-by-minute light exposure patterns to predict sleep metrics and circadian timing (DLMO). Multiple analytical approaches will be employed, including using light (melanopic lux) as an input into a validated mathematical model^{37 40 93} to examine how well light-exposure patterns account for changes in sleep and circadian timing over time within an individual. We will also include light exposure variables (e.g., binned according to phase response curve characteristics⁹⁴) as predictors in a mixed-effects model, with participant random effects, and with circadian timing of sleep (i.e., time between DLMO and sleep onset/offset) as the dependent variable, including other covariates (e.g., age and sex).

For Aim 3, we will use appropriate longitudinal statistical methods, such as latent growth models, to predict the change in sleepiness over time using changes and initial baseline values for sleep metrics, circadian timing and circadian timing of sleep. Wherever appropriate, propensity scores for covariates (e.g., sociodemographics, test anxiety, season) may be included to reduce bias.

Aim 4 will be tested by a parallel approach to aim 3 to predict longitudinal changes in academic performance (WIAT-III scores), using changes in sleep metrics, circadian timing and circadian timing of sleep. To evaluate whether sleepiness acts as a mediator, indirect effects will be calculated as the product of the paths from sleep and circadian metrics to sleepiness, and from sleepiness to the change from Year 7 to Year 9 WIAT-III scores.

Secondary analyses investigating longitudinal relationships between sleep timing, duration and regularity, circadian timing, mental health outcomes and affect, genetics and sociobehavioural factors will also be explored.

Ethics and dissemination

The study has been approved by the Monash University Human Research Ethics Committee (#18718) and the Victorian Department of Education and Training (#2020_004315). Written informed consent will be provided by a parent ('parent' refers to a parent or legal guardian), and written informed assent provided by the participant, via the online e-consent framework implemented in REDCap (The REDCap Consortium, hosted at Monash University^{43 44}). Study findings will be disseminated via scientific publications, scientific conferences, via stakeholders including schools and media.

DISCUSSION

This study will investigate longitudinal relationships between sleep (timing, duration and regularity), circadian timing, environmental light exposure and academic performance. Through a robust longitudinal burst design, and the use of rigorous assessments of sleep-wake timing, circadian timing, and light exposure relevant to the circadian system, this study will provide a valuable dataset suitable for disentangling the relative contributions of sleep, circadian timing and environmental light to the development of cognitive skills and academic achievement in adolescents. These findings will contribute to the understanding of the role of sleep and circadian factors in cognitive development in adolescents. The findings will also provide specific insights into the degree to which light exposure patterns account for observed age-related changes in sleep and circadian timing. Algorithms and markers identified in this study may inform the future development of practical tools to minimise sleep disturbances and sleepiness in healthy adolescents, such as light and sleep-wake monitoring systems. Findings from this study will provide an evidence base to inform child and parent education programs, and inform development of policies and guidelines for improving sleep habits in adolescents.

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Contributors SMWR, BB, JW, AJKP, EK, SWL, MAC and JES designed the study. MR designed the psychological risk protocol for participant safety. JES, AJH, SL and EC adapted procedures during COVID-19 lockdowns. JES drafted the protocol. All authors critically reviewed the protocol and provided comments. All authors approved the final protocol.

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