Cover sheet

Study title	Carryover effects in melatonin suppression
Internal code	
Date of protocol (yyyy-MM-dd)	2025-04-28
Version of protocol	v1.0.0

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Signatures

München, 2025-06-24 _____

Location, date Prof. Dr. Manuel Spitschan

Synopsis

Basic information

Study title	Carryover effects in melatonin suppression
Internal code	
Date of protocol (yyyy-MM-dd)	2025-04-28
Version of protocol	1.0.0

Research question

Light plays a critical role in regulating human circadian rhythms, influencing the secretion of melatonin and cortisol. The retina, beyond its function in vision, serves as a key input for neuroendocrine regulation. The suprachiasmatic nucleus (SCN) acts as the central pacemaker, synchronizing physiological processes in response to environmental light cues. Exposure to artificial light, particularly during evening hours, can significantly alter melatonin suppression, shift circadian rhythms, and impact physiological functions such as core body temperature and glucose metabolism. Recent research highlights that the effects of light on circadian hormones depend on both intensity and spectral composition. Studies indicate that light exposure affects melatonin secretion, but real-world applicability remains limited due to short study durations and disruptions caused by controlled lab environments. An open question is whether preceding light exposure on the day before impacts melatonin suppression. To answer this research question, we will examine carryover effects of light exposure on melatonin suppression by light using a nine-evening in-laboratory pilotstudy.

Study design and protocol

Healthy participants aged 18 to 40 years will be recruited via flyers and digital advertisements for a nine-day in-laboratory pilotstudy. After giving informed consent for screening, participants will receive an online link to complete a questionnaire about their health status to determine eligibility. If they are eligible to participate in the study, they will be invited to the laboratory. If agreeing to proceed, they will give informed consent for the full pilotstudy. Throughout the pilotstudy, they will wear a wrist-worn activity tracking watch, a continuous glucose monitoring (CGM) device on their arm and a lanyard-worn light exposure logger for the entire starting seven days prior to the first evening in the laboratory. In addition, they will complete a daily sleep diary. A pregnancy test for female participants will be performed once at the first visit, in addition to a drug and alcohol test.

On each study evening, participants will be asked to arrive 6.5 hours before their habitual bedtime(HBT), and spend a total of 6 hours in the laboratory, before they leave 30 minutes before their HBT. Upon arrival in the laboratory, they will be provided with a standardised meal (Huel) with a well-balanced macronutrient profile. Then, they will spend 2.5 hours in dim conditions (10 lx). From 2.5 hours before HBT to 0.5 before HBT, they will be exposed to one of two stimuli: a dim condition (10 lx), or a bright condition (1000 lx). The final 0.5 hours before HBT will be spent in dim light (10 lx) again, before they leave the laboratory. Every 30 minutes, participants will give a saliva sample for later melatonin analysis as well as sleepiness rating, and every hour, participants will be performing a psychomotor vigilance test. Additionally, skin temperature will be measured using iButtons attached to the neck and ankle. Core body temperature will be monitored using the eCelsius ingestible capsule system. Participants will continue to wear the wrist-worn activity tracking watch, a continuous glucose monitoring (CGM) device on their arm and a lanyard-worn light exposure logger for a week after completion of the final evening.

Study sample

Inclusion criteria

Domain	Criterion	Assessment method
Age	≥18, ≤40 years of age	Self-report
Biological sex	Men Women with a natural menstrual cycle Women taking monophasic combined oral contraceptives	Self-report
Physical health	Good physical health	Self-report
Mental health	Good mental health	Self-report
Ocular health	Good ocular health	Self-report
Normal colour vision	Normal colour vision	Ishihara-test
Basic English language skills	Ability to comprehend and communicate in basic English	Self-report

Exclusion criteria

Domain	Criterion	Assessment method
Biological sex	Women taking any other type of hormonal contraception	Self-report
BMI	<18 or >30	Measured height and weight
Medication use	Any use of medications	Self-report
Smoking	Habitual smoking	Self-report
Epilepsy	Diagnosis of epilepsy	Self-report
Substance abuse	Excessive alcohol use	AUDIT
Sleep	Poor sleep quality	PSQI >5
Chronotype	Extreme chronotype	MCTQ
Shift work	No shift work in the past 3 months	Self-report
Time zone travel	No inter-time zone travel in the past 3 months	Self-report
Pregnancy	Currently pregnant and < 12 months postpartum	Self-report
Endocrinal alternation	No hormonal imbalances	Self-report
Drug use	Any drug detected	AMP, BZD, COC, MOR/OPI, THC panel (nal von minden GmbH)
Alcohol	Any alcohol in breath	Breathalyzer

Sample size

Due to the exploratory character of this pilot study and resource limitations, we will recruit a total of 16 participants.

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List of abbreviations

AMP	Amphetamines
AUDIT	Alcohol Use Disorders Identification Test
BIA	Bioelectrical impedance analysis
CGM	Continuous Glucose Monitoring
ipRGCs	intrinsically photosensitive retinal ganglion cells
HBT	habitual bedtime
KSS	Karolinska Sleepiness Scale
LEBA	Light Exposure Behaviour Assessment
LED	Light-emitting diode
MCTQ	Munich Chronotype Questionnaire
MHQ	Menstrual History Questionnaire
MOR/OPI	Morphine/Opiates
NSAID	Non-steroidal anti-inflammatory drug
PI	Principle Investigator
FDA	Food and Drug Administration
PSQI	Pittsburgh Sleep Quality Index
M cones	Medium-wavelength-sensitive cones
SCN	Suprachiasmatic nucleus
PLR	Pupillary light reflex
PVT	Psychomotor vigilance test
RHT	Retinohypothalamic tract
THC	Tetrahydrocannabinol

Background

State of the art

Ocular light exposure affects human physiology and behaviour (6). Exposure to light in the evening and at night suppresses the production of the endogenous hormone melatonin in a dose-dependent manner, delays the circadian clock and impacts sleep latency and possibly quality. The 'non-visual' effects of light are mediated by a class of retinal ganglion cells that are intrinsically photosensitive through the photopigment melanopsin, known as intrinsically photosensitive retinal ganglion cells (ipRGCs). Melanopsin is sensitive to short-wavelength light (peak wavelength sensitivity near 490 nm in the living human eye after pre-receptoral filtering by the lens and ocular media). The ipRGCs project to the suprachiasmatic nucleus (SCN) in the hypothalamus via the retinohypothalamic tract (RHT), and via a series of synapses that are then linked to the pineal gland and are responsible for melatonin suppression. Melatonin suppression can be calculated based on light exposure duration, lux intensity using an algorithm (7). Short-wavelength light is associated with alertness, thermoregulation, and heart rate (8).

There are marked individual differences in the impact of light on melatonin suppression, with some individuals being 60x more sensitive than others (9). The reasons underlying this wide individual variability is unclear and cannot be attributed to prior light exposure, which previously has been identified as a modulator for evening sensitivity to light. The day-to-day variability of melatonin production has thus far not been characterised, nor the impact of the modification of light exposure and melatonin suppression on the days before.

Summary of proposed study

This pilotstudy aims to investigate the effects of controlled light exposure on physiological and behavioural outcomes in healthy adults aged 18 to 40 years. Participants will be recruited through flyers and digital advertisements and screened using an online health questionnaire to determine eligibility. Those eligible will provide informed consent and undergo additional screening, including a pregnancy test (if applicable) and drug and alcohol testing.

Participants will undergo a 23-day protocol (7 days pre-laboratory, 9 laboratory days, 7 days post-laboratory). For seven days before their first laboratory session, participants will wear a wrist-worn activity tracker (ActiGraph), a continuous glucose monitoring (CGM) device, and a lanyard-worn light exposure logger (ActLumus), while also completing a daily sleep diary to monitor their habitual sleep patterns and light exposure.

The in-laboratory phase will take place in the climate chamber of the SenseLab, part of the Chair of Building Technology and Climate Responsive Design at the Technical University of Munich (TUM), based on a collaboration for the use of this facility. This phase will span nine consecutive evenings, during which participants will arrive 6.5 hours before their habitual bedtime (HBT) and remain for 6 hours each night, before they leave 30 minutes prior to their HBT. Upon arrival, they will receive a standardized meal (Huel) before spending 2.5 hours in dim light (10 lx). They will then be exposed to either dim (10 lx) or bright (1000 lx) light conditions for two hours, followed by another 30 minutes in dim light (10 lx) before leaving the laboratory. This protocol allows for controlled manipulation of evening light exposure to assess its impact on physiological and cognitive responses.

Throughout each session, saliva samples will be collected every 30 minutes for melatonin analysis, sleepiness and visual comfort ratings will be recorded at the same intervals, and participants will complete a psychomotor vigilance test (PVT) every hour. iButtons will be used to assessskin temperature. The core body temperature will be monitored using an ingestible pill. These measurements will help determine how different light conditions influence circadian rhythms, alertness, and cognitive performance. The findings from this study will contribute to a better understanding of the role of evening light exposure in regulating human physiology and behaviour, with potential implications for optimizing light environments in real-world settings.

Objectives

The objectives of this pilotstudy are:

(1) To characterise inter-daily variability of melatonin production
(2) To characterise inter-daily variability of melatonin suppression by light
(3) To investigate carry-over effects of light-induced melatonin suppression

Study duration

Entire study

The entire pilotstudy will take place over a total of 4 months.

For each participant

Each participant in the pilotstudy will be enrolled for a total of 23 days, comprising an initial 7-day ambulatory monitoring period prior to the first laboratory evening, 9 days of laboratory evenings, and then a final 7-day period of ambulatory monitoring.

Study sample

Description of study sample

In this pilotstudy, we will recruit healthy participants aged 18-40 years with no systematic, ocular and retinal diseases, no psychiatric or neurological diseases, with normal sleep-wake behaviour, normal colour vision, and meet the following additional criteria:

Inclusion criteria

Domain	Criterion	Assessment method
Age	≥18, ≤40 years of age	Self-report
Biological sex	Men	Self-report
	Women with a natural menstrual cycle	
	Women taking monophasic combined oral contraceptives	
Physical health	Good physical health	Self-report
Mental health	Good mental health	Self-report
Ocular and retinal health	Good ocular and retinal health	Self-report

Short physical examination	Good health	Physicians
Normal colour vision	Normal colour vision	Ishihara plates
Basic English language skills	Ability to comprehend and communicate in basic English	Self-report

Exclusion criteria

Domain	Criterion	Assessment method
Biological sex	Women taking any other type of hormonal contraception	Self-report
BMI	<18 or >30	Calculation from measured height and weight
Medication use	Any use of medications	Self-report
Smoking	Habitual smoking	Self-report
Epilepsy	Diagnosis of epilepsy	Self-report
Substance abuse	Excessive alcohol use	AUDIT
Sleep	Poor sleep quality	PSQI >5
Chronotype	Extreme chronotype	MCTQ
Shift work	No shift work in the past 3 months	Self-report
Time zone travel	No inter-time zone travel in the past 3 months	Self-report
Pregnancy	Currently pregnant and < 12 months postpartum	Self-report
Endocrinal alteration	No hormonal imbalances	Self-report
Drug use	Any drug detected	AMP, BZD, COC, MOR/OPI, THC panel (nal von minden GmbH)
Alcohol	Any alcohol in breath	Breathalyzer

Recruitment

We will recruit participants through a variety of means. This will include a multi-modal strategy using posters, ads placed on the internet and in public, mailing list postings, the recruitment system SONA, word-of-mouth and invitation via the WhatsApp group of health and sports science students. Participants will be sent the participant information sheet with the informed consent form and will have the opportunity to participate in an information session demonstrating the lab and devices used in the pilotstudy prior to enrolling in the pilotstudy.

Sample size

In this pilotstudy, we will be following a strong within-subjects design, where each participant participates in a series of conditions, including serving as their own control. Acknowledging the exploratory character of this study, we expect to run it with a total sample size of 16 participants.

Protocol

Overview

The pilotstudy has four components: Screening, ambulatory monitoring before the in-laboratory session (7 days), a 9-day sequence of laboratory sessions during the evening, and final ambulatory monitoring (7 days) after the in-laboratory sessions. The screening aims to identify participants and

evaluate their suitability for the pilotstudy against a series of selection criteria. Once enrolled in the pilotstudy, participants will attend evening in-laboratory measurements and assessment on nine sequential days. The pilotstudy will take place in English.

Location

In the context of a collaboration with the Chair of Building Technology and Climate Responsive Design at the Technical University of Munich (TUM), all screening and in-laboratory visits will take place at the TUM SenseLab at the main campus

Screening

Demographic variables

At screening, we will ask participants for their age, sex and gender identity.

Health variables

Physical health will be assessed using a self-report. We will ask participants whether they are taking any medication, are smoking or have a diagnosis of epilepsy or having any hormonal imbalances. Additionally female participants will be asked about their menstrual health (MHQ),the specific type of monophasic combined oral contraceptive they are taking (if applicable) and fill out a reproductive status questionnaire. We will use an urine based pregnancy test at the beginning of the first in laboratory visit.

Questionnaires

During screening, participants will complete a series of questionnaires, delivered via the online platform REDCap on a server set up and maintained by the Chronobiology & Health team at TUM and will be acquainted via the app momenTUM. The server is set up as a virtual machine hosted by the Leibniz-Rechenzentrum der Bayerischen Akademie der Wissenschaften.

Health survey

We will assess basic demographic and relevant health data from the participants.

Alcohol Use Disorders Identification Test (AUDIT)

Participants will complete the Alcohol Use Disorders Identification Test (AUDIT), an instrument to examine substance and alcohol abuse, in the self-report version.

Pittsburgh Sleep Quality Index (PSQI)

Participants will complete the Pittsburgh Sleep Quality Index (PSQI), an instrument to determine sleep quality.

Munich Chronotype Questionnaire (MCTQ)

Participants will complete the Munich Chronotype Questionnaire (MCTQ) to determine their chronotype.

Light Exposure Behaviour Assessment (LEBA)

Participants will complete the Light Exposure Behaviour Assessment (LEBA) instrument for determining habitual light exposure patterns.

In-laboratory measurements

Participants will come to laboratory 6,5 hours prior to their HBT and leave the laboratory 30 minutes before their HBT. Their HBT will be established using the information given at screening. From three days before the pilotstudy, participants will abstain from non-steroidal anti-inflammatory drug (NSAID)

and alcohol intake. On the day of the pilotstudy, participants will be asked to abstain from caffeine intake four hours after their habitual waketime and avoid acute physical activity. Four participants will be tested at a time.

Anthropometric measurements

At the beginning of the first visit, we will measure participant height and weight.

Alcohol and THC test

At the beginning of each laboratory evening, participants will be tested for alcohol and THC use. Participants will be asked to use a Breathalyzer (Alkoholtester ACE A) to determine blood alcohol content (BAC). Participants will also be asked to produce a urine sample in a collection device, which will then be tested using a multi-drug panel. Should either test be positive, participants will be removed from the pilotstudy.

Saliva

During the in-laboratory measurements, saliva will be sampled from participants every 30 minutes in order to analyse melatonin levels.

Continuous glucose monitoring

Participants will wear the Freestyle Libre Pro (Abbott Laboratories), a Continuous Glucose Monitoring (CGM) device, for continuous 24-hour glucose level monitoring.

Skin temperature

iButtons will be attached to the neck and ankle of participants during their visit of the laboratory to record skin temperature.

Core body temperature

Participants will ingest a pill, utilizing the Body Cap eCelsius system, to measure core body temperature. The device is CE certified, and data will be stored locally. To ensure participant safety, they will wear a wristband to prevent entry into an MRI environment until the pill has been naturally expelled. The pill is expected to be expelled within 24-48 hours following ingestion.

Light stimuli

Stimulus presentation

We will study two distinct light exposure conditions over a five-hour period in each lab session, presented on different study days. Each session will begin with 2.5 hours in dim light (10 lx), followed by 2 hours of exposure to either dim light (10 lx) or bright light (1000 lx). The session will conclude with 30 minutes in dim light (10 lx) before departure. Stimuli will be delivered using room illumination or specialized light sources.

Core body temperature

We will continuously measure participants' core body temperature using the BodyCap e-Celsius system, starting shortly after they finish their standardized dinner in the laboratory. This system involves the use of an ingestible temperature pill, which connects to an external device via RFID and measures corebody temperature. The device is CE-certified and data will be stored locally. Participants will eject the pill with 24-48 hours of ingestion. As the device is not MRI safe, participants will receive a wristband indicating that they cannot enter an MRI scanner. The participants will be asked to wear a certain type of clothing to ensure the same clothing factor of 0.5, which means they should wear normal underwear, long sleeves with light material, long trousers or skirts made of

normal/light material (excluding jeans). They should also wear regular short socks (not stockings or tights) and regular dress or sports shoes (no heavy boots).

Visual comfort

Every 30 minutes, participants will complete a brief visual comfort questionnaire delivered on digital tablets or via pen and paper.

Karolinska Sleepiness Scale (KSS)

This scale measures the subjective level of sleepiness at a particular moment in time via self-reporting. It will be delivered via digital tablets or pen and paper.

Psychomotor vigilance test (PVT) attention test

Every 60 minutes, participants will perform an auditory psychomotor vigilance test (PVT) using the PVT-192 Psychomotor Vigilance Task Monitor (Ambulatory Monitoring, Inc.). This test measures sustained attention and reaction time. It can be ideal for assessing alertness changes due to sleep deprivation or light exposure.

Salivary melatonin

Every 30 minutes, participants will give saliva samples throughout the protocol. Upon collection, these samples will be centrifuged and frozen.

Participant monitoring during in-laboratory sessions

During the study period, participants are kindly requested to minimize their movements and, if possible, maintain the same sitting position for the majority of the time.

Timeline

For each in-laboratory visit, participants will arrive in the laboratory 6.5 hours before their HBT. They will firstly be reminded of the instructions and the requirements to participate in the session especially about sustained participations and signing an informed consent.

During the first hour of the visit, they will complete a breathalyser, to ensure they are not under the influence of alcohol or THC. Then, they will be provided with a standardised meal (Huel, which has a well-balanced macronutrient profile and portion sizes will be adjusted based on participants metabolic requirements following the Mifflin-St. Jeor formula) and then ingest the pill which is part BodyCap e-Celsius system to initialise the core body temperature measures. In addition, iButtons will be attached to their neck and ankle.

The experiment will begin 5.5 hours before their habitual bedtime. Participants will sit on a couch or chair while they provide an initial saliva sample and then will complete a battery of behavioural tests that includes a sleepiness questionnaire (KSS), a visual comfort questionnaire, and a Psychomotor Vigilance Test (PVT). After this, the lights in the room will be set to a dim state (10 lux). This light stimulus will be maintained for the 2.5 hours of the experiment, before a 2-hour period of different exposures (10 lux or 1000 lux). Every 30 minutes, participants will again provide a saliva sample and complete the behavioural test of sleepiness and alertness (every 30 min) and attention test (every 60 min.)

They will be offered breaks every 30 minutes after the tests and will be allowed toilet breaks whenever needed, where they will wear blue light blocking glasses. Throughout this part of the experiment, except when completing the battery of tests, participants will be allowed to read printed materials and/or listen to pre-selected audiobooks or musical albums.

The participants will depart from the lab 30 minutes before their HBT. The next day, the participants will come back 6.5 hours before there HBT, and the same process will be completed on each evening.

The light exposure sequence will be selected to correspond to a 2-label, 3-level counterbalanced De Bruijn sequence. In this sequence, all transition 2-back triplets occur once, i.e. [Dim, Dim, Dim]; [Dim, Dim, Bright]; [Dim; Bright; Bright] and so on. There are two possible 2-label, 3-level DeBruijn sequences:

Day	Sequence A	Sequence B
Day 1	Dim (10 lx)	Dim (10 lx)
Day 2	Dim (10 lx)	Bright (1000 lx)
Day 3	Dim (10 lx)	Bright (1000 lx)
Day 4	Bright (1000 lx)	Bright (1000 lx)
Day 5	Bright (1000 lx)	Dim (10 lx)
Day 6	Bright (1000 lx)	Dim (10 lx)
Day 7	Dim (10 lx)	Dim (10 lx)
Day 8	Bright (1000 lx)	Bright (1000 lx)
Day 9	Dim (10 lx)	Dim (10 lx)

Participants will be tested in groups of 4 participants. Each group will be randomly assigned one sequence.

Sampling of biological materials

Saliva

Saliva will be sampled every 30 minutes using Salivette collection devices. These devices are standard for saliva collection and later assay. In brief, participants chew on a cotton swab for 5 minutes and place it into a plastic collection tube. The salivette will then be stored for later processing.

The saliva will be assayed for melatonin using enzyme-linked immunosorbent assay (ELISA). The assaying will be performed by the bioanalytics company NovoLyTiX. The samples will be destroyed after completion of the study. All samples will be retained securely until the study has been completed and all data have been published.

Instruments

In this pilotstudy, we will use various instruments for visual stimulation and measurements. The following table gives the devices, the manufacturer, their purpose, and any certification status.

Device	Manufacturer	Purpose	Certification
ActLumus	Condor (São Pãolo, Brasil)	Measurement of rest-activity cycles and light exposure	IEC60601-1:2006, IEC60601-1-2:2007, IEC60601-1-11:2010

PVT-192 Psychomotor Vigilance	CWE, Inc.	Reaction Time	Class I, Self -Certified
GT3X	ActiGraph, LLC	Measurement of rest-activity cycles	–
Freestyle Libre Pro	Abbott	Continuous Glucose Monitoring	FDA
e-Celsius	BodyCap	Measurement of core body temperature using an ingestible pill	CE
Skin temperature, iButton	Analog Devices	Recording skin temperature using iButtons attached to the neck and ankle.	DS1922L-F5

Appropriate certificates are appended in Appendix A. All devices were purchased from internal funds of the investigator and were not sponsored by the manufacturer(s).

Statistical analysis plan

Overview

The statistical analysis will assess the effects of light exposure on circadian and metabolic physiology, focusing on melatonin suppression, psychomotor vigilance, core body temperature, skin temperature, , glucose metabolism, and subjective sleepiness. Analyses will follow a within-subjects design, leveraging the counterbalanced De Bruijn sequence to minimize order effects.

Main Hypotheses

In line with the objectives of this study, three main hypotheses can be proposed.

Hypothesis	Outcome measure	Statistical test
H1: Light exposure (1000 lx) leads to greater melatonin suppression compared to dim light (10 lx)	Salivary melatonin concentration (pg/mL)	Linear mixed-effects model (LMM) with condition (dim vs. bright) and time as fixed effects, participant as a random effect
H2: The impact of light exposure on melatonin suppression is modulated by prior light history	Preceding day's light exposure (lux-hour, logged from wearable light logger)	LMM with preceding light exposure as a covariate
H3: Individual variability in melatonin suppression is significant	Individual differences in suppression slope (Δ melatonin per lx exposure)	Variance component analysis in LMM; hierarchical clustering to stratify sensitivity subgroups.

Secondary Hypotheses

Additionally, the following secondary hypotheses will be tested.

Hypothesis	Outcome measure	Statistical test
H4: Bright light exposure reduces subjective sleepiness compared to dim light	Karolinska Sleepiness Scale (KSS) scores	Repeated-measures ANOVA with light condition and time as factors
H5: Bright light exposure enhances psychomotor vigilance	Psychomotor Vigilance Test (PVT) reaction time (ms), PVT lapses (RT > 500 ms)	Paired t-tests / LMM with time and condition as fixed effects
H6: Core body temperature follows a different trajectory under bright vs. dim light	Body temperature (°C) measured via ingestible sensor	Generalized Additive Model (GAM) to model non-linear trends
H7: Distal-proximal skin temperature gradient follows a different trajectory under bright vs. dim light	Difference between distal and proximal temperature (°C) measured on the skin	Generalized Additive Model (GAM) to model non-linear trends
H8: Bright light exposure influences glucose metabolism	CGM-derived glucose levels (mg/dL), Glucose variability metrics (e.g., standard deviation, MAGE)	LMM with light condition as a fixed effect, participant as a random effect

Sample size justification

Based on prior studies (Phillips et al., 2019; Higuchi et al., 2013), we expect an effect size of d = 0.8 for melatonin suppression. Due to the exploratory charactery of this study and due to resource limitations, we will aim of 16 participants. Due to the strong within-subjects design, we expect that the results of this pilotstudy will be informative.

Participant remuneration

Participants will be remunerated for their time, with a rate of €10 for each evening session. If they complete all sessions, they will receive a bonus of up to €450, leading to a total maximum compensation of €540. If participants drop out of the study, no bonus will be paid.

Risks and benefits

Participants may find adhering to a regular sleep-wake cycle for the nine-day study period challenging. To minimize this risk, our screening process identifies individuals who are likely to maintain a stable sleep schedule without difficulty.

Spending nine consecutive evenings in the laboratory may cause discomfort or fatigue. However, our research team will closely monitor participants throughout all lab visits and provide necessary support if they report any distress.

Wearing multiple devices, including an actigraph tracker, light logger, and continuous glucose monitoring (CGM) devices, for an extended period may be inconvenient or uncomfortable. The CGM device requires a small sensor to be inserted under the skin, which may cause mild discomfort or irritation. Participants will receive detailed instructions on proper sensor placement, care, and troubleshooting, and the research team will be available to assist with any issues.

The CGM device may also cause mild skin irritation, infection risk at the insertion site, or sensor adhesion issues. To minimize these risks, participants will be provided with proper cleaning instructions, and sensor replacement will be available if necessary.

Participants may also experience mild discomfort from repeated saliva sampling throughout the pilotstudy. However, saliva collection is a non-invasive method for assessing melatonin levels, making it a safer and less burdensome alternative to blood sampling.

Some participants may find frequent saliva collection inconvenient or experience dry mouth. To mitigate this, participants will be informed whether water intake is allowed before sampling to reduce discomfort.

Repeated evening visits and sleep schedule restrictions may lead to mental and physical fatigue. To address this, participants will be offered rest breaks as needed, and fatigue levels will be monitored throughout the pilotstudy. The research team will intervene if excessive fatigue is reported.

Despite these risks, the pilotstudy offers significant benefits by advancing our understanding of how evening light exposure influences circadian physiology, neuroendocrine function, and metabolic regulation. This research has potential implications for sleep health, workplace lighting recommendations, and clinical applications for individuals with circadian rhythm disorders or metabolic conditions. Participants may also gain insight into their own sleep patterns and physiological responses to light, which could help inform healthier lifestyle habits.

Overall, we have carefully evaluated the potential risks and benefits to ensure participant safety while maximizing the scientific value of the study.

Data protection

Legal consent

Prior to any data collection, participants will be informed of how their data are processed and will have ample opportunities to ask questions. Participants will receive paper consent forms and detailed information about processing of their personal data, which will include their name and signature. These consent forms (see attached Legal *Declaration of Consent* including *Information Sheet* in accordance with the EU General Data Protection Regulation) will be retained in a locked cabinet at TUM. Only selected people will have keys to this cabinet. Upon the start of their participation, in-lab subjects are assigned a random subject ID number. This subject number will be used to label data obtained on the task. At no point will the subjects' names be tied to their subject number. There will be no method to go from subject ID number to subject name or match subject responses on the task to subject identity.

Pseudonymization

At enrolment of the pilotstudy, participants will be assigned a pseudonym participant ID which is necessary to ensure scheduling of appointments and planning of logistics of participation, will be stored in password-protected and encrypted spreadsheet in a restricted TUM location. The linkage list between name and participant ID, the password will only be known to the experimenter and PI and cannot be read, copied, modified or removed by unauthorized persons.

All data collected in this project will be labelled using the pseudonyms and stored directly in a pseudonymised form. I.e. the data will be collected under a numerical ID without a reference to contact details and processed without any assignment to personal data of the participants.

The documentation of data and its archiving occurs in a pseudonymised form in a protected electronic database, to which only a limited number of authorised employees have access, including here

doctoral students, who are obligated to professional and data secrecy. This data secrecy obligation will also continue to exist after termination of their employment.

Processing of personal data during the study

The processing of personal data will be carried out in such a way that the data can no longer be attributed to a data subject without the use of additional information. The additional information is kept separately and is subject to appropriate technical and organizational measures. Once data have been collected either in-laboratory or online, they are stored on secure, password-protected lab computers and server space accessible only to trained laboratory personnel.

Collected data may be used for the preparation of anonymised scientific research work and may also be published and used in an anonymised form in medical journals and scientific publications, so that a direct reference to participant person cannot be established.

Data analysis

The collected and saved data will be classified as health data under the "very high" protection level. The transfer of the analysis from the laboratory will be done pseudonymized using participant ID, so the laboratory cannot make a connection between the person and samples. Furthermore, the sample analysis results will be delivered in encrypted form to the study leader at TUM. All participating laboratory physicians and employees are subject to medical confidentiality as stipulated by German law.

List of data types

Type of data	Location	Note
Participant name	Informed consent forms	Forms will be locked in a storage cabinet
Participant signature		
Name and participant ID linkage list	Password-protected and encrypted spreadsheet on shared network drive accessible only to project personnel	Only the project team have access
Sleep diaries collected in sleep-wake stabilisation period	Data collected on password-protected storage server	All digital data will be pseudonymised and labelled with participant ID
Demographic data, including age, sex, gender identity and Questionnaire results	Data collected on password-protected storage server	
Saliva samples	Freezers at TUM, shipped to bioanalytics facilities	Saliva samples will be pseudonymised and labelled with participant ID
Melatonin concentrations obtained from saliva samples	Spreadsheets and data files on shared network drive accessible only to project personnel	All digital data will be pseudonymised and labelled with participant ID
Data from wrist-worn activity tracker		
Data from wearable light logger		
Performance data from PVT		
Data from iButtons		

Participant information sheet and informed consent form

The participant information sheet and informed consent form is attached to the application.

Study materials

All study materials are attached to the ethics application (Appendix B).

Appendix A

2023.05.04_AppendixA_ABPMpro.pdf

2023.05.04_AppendixA_ActiGraphGT3X.pdf

2023.05.04_AppendixA_ActTrust.pdf

2023.05.04_AppendixA_BodyCap.pdf

2023.05.04_AppendixA_Fibion.pdf

2023.05.02_AppendixA_Freestyle_Libre_Pro.pdf

2023.05.04_AppendixA_i-Button.pdf

2023.05.04_AppendixA_OMRON.pdf

2023.05.04_AppendixA_Tobii3pro.pdf

2023.05.04_AppendixA_U-Rhythm.pdf

2023.05.04_AppendixA_SOP_Cleaning_U-RHYTH_.pdf

2023.05.04_AppendixA_SOP_Reporting_sampling_outcome.pdf

2023.05.04_AppendixA_SOP_sample_retrieval.pdf

2023.05.04_AppendixA_SOP_Starting_to_sample_with_U-RHYTHM.pdf

2023.05.04_AppendixA_SOP_U-RHYTHM_Checklist_and_log_generic.pdf

2023.05.04_AppendixA_SOP_ Ending_U-RHYTHM_sampling_session.pdf

Appendix B

2023.05.04_AppendixB_ASHRAE-en.pdf

2023.05.04_AppendixB_AUDIT-en.pdf

2023.05.04_AppendixB_Demographics-en.pdf

2023.05.04_AppendixB_Device_Tolerability-en.pdf

2023.05.04_AppendixB_MCTQ-en.pdf

2023.05.04_AppendixB_MHQ-en.pdf

2023.05.04_AppendixB_MoodandVisualcomfort-en.pdf

2023.05.04_AppendixB_KSS-en.pdf

2023.05.04_AppendixB_LEBA-en.pdf

2023.05.04_AppendixB_PSQI-en.pdf

2023.05.04_AppendixB_ReproductiveQ-en.pdf

2023.05.04_AppendixB_SleepDiary-en.pdf

References

1. Upton TJ. U-RHYTHM microdialysis: Towards ambulatory metabolodynamics: University of Otago; 2021.
.
5.
6. Blume C, Garbazza C, Spitschan M. Effects of light on human circadian rhythms, sleep and mood. Somnologie (Berl). 2019;23(3):147-56.
7. Giménez MC, Stefani O, Cajochen C, Lang D, Deuring G, Schlangen LJ. Predicting melatonin suppression by light in humans: Unifying photoreceptor-based equivalent daylight illuminances, spectral composition, timing and duration of light exposure. Journal of Pineal Research. 2022;72(2):e12786.
8. Cajochen C, Munch M, Kobialka S, Krauchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. The journal of clinical endocrinology & metabolism. 2005;90(3):1311-6.
9. Phillips AJK, Vidafar P, Burns AC, McGlashan EM, Anderson C, Rajaratnam SMW, et al. High sensitivity and interindividual variability in the response of the human circadian system to evening light. Proc Natl Acad Sci U S A. 2019;116(24):12019-24.
10. Rodgers J, Peirson SN, Hughes S, Hankins MW. Functional characterisation of naturally occurring mutations in human melanopsin. Cell Mol Life Sci. 2018;75(19):3609-24.
11. Chang AM, Duffy JF, Buxton OM, Lane JM, Aeschbach D, Anderson C, et al. Chronotype Genetic Variant in PER2 is Associated with Intrinsic Circadian Period in Humans. Sci Rep. 2019;9(1):5350.
12. Roecklein K, Wong P, Ernecoff N, Miller M, Donofry S, Kamarck M, et al. The post illumination pupil response is reduced in seasonal affective disorder. Psychiatry Res. 2013;210(1):150-8.
13. Higuchi S, Hida A, Tsujimura S, Mishima K, Yasukouchi A, Lee SI, et al. Melanopsin gene polymorphism I394T is associated with pupillary light responses in a dose-dependent manner. PLoS One. 2013;8(3):e60310.
14. Smieszek SP, Polymeropoulos CM, Birznieks G, Polymeropoulos MH. Case report: A novel missense variant in melanopsin associates with delayed sleep phenotype: Whole genome sequencing study. Front Genet. 2022;13:896192.
15. Gutierrez-Amavizca BE, Prado Montes de Oca E, Gutierrez-Amavizca JP, Castro OD, Ruiz-Marquez CH, Perez Conde-Andreu K, et al. Association of P10L Polymorphism in Melanopsin Gene with Chronic Insomnia in Mexicans. Int J Environ Res Public Health. 2021;18(2).
16. Lee SI, Hida A, Kitamura S, Mishima K, Higuchi S. Association between the melanopsin gene polymorphism OPN4*Ile394Thr and sleep/wake timing in Japanese university students. J Physiol Anthropol. 2014;33:9.