# Cover sheet

|  |  |
| --- | --- |
| **Study title** | Visual gamma stimulation in sleep |
| **Internal code** | Gamma\_Sleep |
| **Date of protocol (yyyy-MM-dd)** |  |
| **Version of protocol** | v1.0 |

|  |  |
| --- | --- |
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# Signatures

Munich, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Location, date Prof. Dr. Manuel Spitschan

# Synopsis

## Basic information

|  |  |
| --- | --- |
| **Study title** | Visual gamma stimulation in sleep |
| **Internal code** | Gamma\_Sleep |
| **Date of protocol (yyyy-MM-dd)** |  |
| **Version of protocol** | v1.0 |

## Research question

Light influences human physiology and behaviour and modifies the production of the endogenous hormone melatonin. This is due to a pathway originating in the retina. How exactly different stimuli impact on human melatonin production are not known. Here, we will examine how stimuli presented differently to the two eyes affect melatonin suppression and how this depends on the spatial and temporal frequency of the stimuli.

## Study design

Participants will be participating in four in-laboratory sessions over a total study period of 5 weeks, including an initial circadian stabilisation period. Participants will spend a total of 6 hours in the laboratory during which they will repeatedly give saliva samples for later melatonin assays, complete reaction time and other rating tests. Throughout each session, their pupil size, core body temperature and other physiological parameters will be monitored. In the 1.5 hours preceding their habitual bed time until 1 hour after habitual bed time, participants will be exposed to one of multiple possible stimuli. The same participant will be exposed to four different stimuli: (A) dim light, (B) constant light, and two active stimulus conditions (C & D). The temporal frequency and spatial properties of the stimulus, and the extent to which stimuli are presented between the two eyes will vary between different participants.

## Study sample

### Inclusion criteria

|  |  |  |  |
| --- | --- | --- | --- |
| **Domain** | **Criterion** | **Assessment method** | |
| Age | ≥18, ≤40 years of age | Self-report | |
| Physical health | Good physical health | Self-report | |
| Mental health | Good mental health | Self-report |
| Ocular health | Good ocular health | Ophthalmological examination by ophthalmologist |
| Binocular vision |  | Titmus Fly Test | |
| Visual acuity | Normal or corrected-to-normal visual acuity | Landolt C | |
| Colour vision | Normal colour vision | Cambridge Colour Test, anomaloscope | |
|  |  |  | |

### Exclusion criteria

|  |  |  |
| --- | --- | --- |
| **Domain** | **Criterion** | **Assessment method** |
| 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 | MEQ |
| 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 |
|  |  |  |

## Sample size and sample size calculations

We will recruit a total of 48 participants.

## Protocol in brief

The study has a serious of components, separated into screening, pre-laboratory measurements and laboratory measurements. During screening participants will complete a series of questionnaires and assessments. Upon inclusion in the study, participants will complete a total of four in-laboratory sessions. Participants will come to the laboratory approx. 5 hours prior to their habitual bedtime and stay until approx. 1 hour after their habitual bedtime. In the 1.5 hours preceding their habitual bed time until 1 hour after habitual bed time, participants will be exposed to one of multiple possible stimuli. The same participant will be exposed to four different stimuli: (A) dim light, (B) constant light, and two active stimulus conditions (C & D). Throughout the entire evening protocol, we will record their saliva every hour and record various subjective ratings. We will record various physiological parameters continuously.

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# List of abbreviations

|  |  |
| --- | --- |
| AUDIT | Alcohol Use Disorders Identification Test |
| CCT | Cambridge Colour Test |
| CSF | contrast sensitivity function |
| EDTA | ethylenediamine tetraacetic acid |
| HMD | head-mounted display |
| ipRGCs | intrinsically photosensitive retinal ganglion cells |
| IR | infrared |
| KSS | Karolinska Sleepiness Scale |
| L cones | Long-wavelength-sensitive cones |
| LED | Light-emitting diode |
| MCTQ | Munich Chronotype Questionnaire |
| mEDI | melanopic equivalent daylight illuminance |
| MEQ | Morningness-Eveningness Questionnaire |
| PSQI | Pittsburgh Sleep Quality Index |
| M cones | Medium-wavelength-sensitive cones |
| S cones | Short-wavelength-sensitive cones |
| SCN | suprachiasmatic nucleus |
| SNP | single-nucleotide polymorphism |
| SSS | Stanford Sleepiness Scale |
| OPN4 | *Opsin 4* (melanopsin) gene |
| PER2 | *Period Circadian Regulator 2* gene |
| PLR | pupillary light reflex |
| PVT | psychomotor vigilance test |
| RHT | retinohypothalamic tract |

# Background

## State of the art

Ocular light exposure affects human physiology and behaviour [1]. 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 on 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, the so-called 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 synpases are then linked to the pineal gland responsible for melatonin suppression.

Which aspects of light can stimulate the RHT are under active investigation, with recent research on spectrum, intensity, colour temperature and temporal patterning of pulses being available. However, how information from the two eyes is integrated in the RHT is largely unknown. In a previous analysis of existing data combining data points from different sources, we found that melatonin suppression is almost 10x more sensitive under binocular (two-eyed) viewing compared to monocular viewing [2]. This points to a possible nonlinear integration of binocular information. The temporal sensitivity of melatonin suppression is largely unknown. We previously investigated how light flicker sinusoidally at different temporal sensitivities controls pupil size, but no such data exist for melatonin suppression.

There are marked individual differences in the impact of light on melatonin suppression, with some individuals being 60x more sensitive than others [3]. 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. There are also genetic variations, which may influence light sensitivity, both in the gene coding for melanopsin (OPN4) [4], and in a gene associated with the circadian clock (PER2) [5]. How these genes map onto the phenotypic variability of light exposure is currently not known.

## Summary of proposed study

Here, we examine binocular integration in the non-visual effects of light as a function of temporal sensitivity using (A) no/dim light, (B) constant light, (C) in-phase dichoptic flickering and (D) counter-phase dichoptic sinusoidally flickering stimuli in a controlled 6-hour evening light exposure protocol. Stimuli will be presented in wide-field viewing conditions using a virtual-reality (VR) head-mounted display (HMD) with integrated pupillometry and eye-tracking capabilities. Flicker frequency will be varied across participants. While in some conditions, the stimuli will be homogenous fields, other conditions will involve spatial patterns. Conditions (A)-(D) will be presented as a within-subjects factor, with participants repeatedly visiting the laboratory while their sleep-wake cycle is held regular through instruction and monitoring using non-invasive wrist-worn actigraphy. While in the laboratory, a series of (psycho)physiological parameters will be measured in response to the light stimuli: (1) eye movement behaviour (fixation, eye closure), (2) pupillometry (pupil size, dynamic responses to pupil size), (3) core body temperature using an ingestible temperature pill, (4) psychophysical performance, (5) visual comfort, (6) mood, (7) brain activity as measured with electroencephalography, (8) salivary melatonin.

## Reasons for proposed study

At present, we do not understand how information from the two eyes is integrated in the non-visual pathways underlying suppression. This study illuminates the biological capacity of the RHT to respond to light. How environmental stimuli such as light control our physiology is key to optimising the use of light in the built environment.

# Objectives

The goal of this study is to examine and characterise the impact of light on human neuroendocrine physiology and cognition. Specifically, we will:

1. Characterise the impact of different stimulation paradigms (monocular, binocular) on non-visual physiology
2. Characterise the impact of spatial frequency of visual stimulation on non-visual physiology
3. Characterise the impact of temporal frequency of visual stimulation on non-visual physiology

These specific aims will be approached through a series of well-controlled laboratory studies in which participants will be presented with a range of visual stimuli that will be modulated parametrically in space and time.

# Study duration

## Entire study

The entire study will take place over a total of 5 months.

## For each participant

Each participant participating in the study will be enrolling for a total of 5 weeks, consisting of a total of 4 in-laboratory visits.

# Study sample

## Description of study sample

In this study, will recruit and enrol healthy participants aged 18-40 years of age with no systematic, ocular and retinal diseases, no psychiatric or neurological diseases, with normal sleep-wake behaviour, normal colour vision, and

## Inclusion criteria

|  |  |  |
| --- | --- | --- |
| **Domain** | **Criterion** | **Assessment method** |
| Age | ≥18, ≤40 years of age | Self-report |
| Physical health | Good physical health | Self-report |
| Mental health | Good mental health | Self-report |
| Ocular health | Good ocular health | Ophthalmological examination by ophthalmologist |
| Binocular vision |  | Titmus Fly Test |
| Visual acuity | Normal or corrected-to-normal visual acuity | Landolt C |
| Colour vision | Normal colour vision | Cambridge Colour Test, anomaloscope |
|  |  |  |

### Exclusion criteria

|  |  |  |
| --- | --- | --- |
| **Domain** | **Criterion** | **Assessment method** |
| 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 |
| Recreational drug use | Use of recreational drugs | Self-report |
| Substance abuse | Excessive alcohol use | AUDIT |
| Sleep | Poor sleep quality | PSQI >5 |
| Chronotype | Extreme chronotype | MEQ |
| 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 |
|  |  |  |

## Recruitment

We will recruit participants through a variety of means. This will include a multi-modal strategy using fliers, posters, advertisements placed in local newspapers and other outlets, as well as ads placed on the internet. Additionally, participants will be recruited through the MPI Experiment Participant Recruiting System *banto*.

## Sample size

In this study, we will be following a strong within-subjects design, where each participant participates in a series of conditions, including serving as their own control. We expect to run this study in a total of 48 participants.

# Protocol

## Overview

The study has a series of components, spread into screening, pre-laboratory measurements, and laboratory measurements. The purpose of screening is to identify participants and evaluate their suitability for the study against a series of selection criteria. Once enrolled in the study, participants will complete a series of pre-laboratory measurements, including actigraphy and sleep-wake stabilisation, which they will complete from one week prior to the first session to the last session.

## 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, along with an examination by clinical study staff. We will ask participants whether they are taking any medication, are smoking or have a diagnosis of epilepsy.

### 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. The server is set up as a virtual machine hosted by the Leibniz-Rechenzentrum der Bayerischen Akademie der Wissenschaften.

#### Munich Chronotype Questionnaire (MCTQ)

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

## In-laboratory measurements

Participants will come to laboratory 5 hours prior to their habitual bedtime and leave the laboratory 1 hour after their behavioural bedtime. Their habitual bedtime will be established using the information given at screening. From three days before the study, participants will abstain from non-steroidal anti-inflammatory drug (NSAID) and alcohol intake. On the day of the study, participants will be asked to abstain caffeine intake and avoid acute physical activity. Three participants will be tested at a time.

### Light stimuli

#### Stimulus presentation

We will examine four stimuli: (A) no light (dark), (B) constant light at the half-max luminance available in virtual-reality head-mounted display HTC Vive Pro Eye, and two active stimulus conditions (C) and (D). Stimuli will be wide-field. A fixation cross will be displayed in the centre of the screen to avoid fixational eye movements. Stimuli will vary parametrically in temporal and spatial frequency.

## Timeline

For each in-laboratory visit, participants will arrive to the lab 5 hours before their habitual bedtime. They will firstly be reminded of the instructions and the requirements to participate in the session.

During the first hour of the visit, they will complete a breathalyser and urine test, to ensure they are not under the influence of alcohol or THC. Then, they will complete a short calibration procedure for the Tobii binocular eye tracker of the HTC Pro Eye that involves wearing the VR headset and fixating on different targets that appear on the screen. Finally, they will ingest the pill that is part BodyCap e-Celsius system to initialise the core body temperature measures.

The experiment will begin 4 hours before their habitual bedtime. Participants will sit in armchairs placed in individual booths. They will provide an initial saliva sample, and they will complete a battery of behavioural tests that includes: a sleepiness questionnaire, a mood questionnaire, a visual comfort questionnaire, and a 2-minute auditory 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 initial 2 hours of the experiment. Every 30 minutes, participants will again provide a saliva sample and complete the battery of behavioural tests mentioned previously. They will be offered breaks every 30 minutes after the tests and will be allowed toilet breaks whenever needed. 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.

After 2 hours in dim light, the light intervention will begin (2 hours before habitual bedtime). Participants will wear the HTC Pro Eye headsets and will view one of the following conditions: i) dark constant background, ii) bright constant background, iii) dichoptic in-phase flicker, iv) dichoptic counter-phase flicker. The frequency of the flicker conditions will be either 0.05 Hz, 0.5 Hz, 5 Hz. The different conditions will be randomised within-subjects, and the flicker frequency will be randomised between-subjects. Participants will wear the HTC Pro Eye headsets for 20 minutes at a time, and while wearing it, will complete a modified visual psychomotor vigilance test (PVT). Their pupil size and eye movement behaviour will be recorded with the Tobii binocular eye tracker of the HTC Pro Eye. Every 30 minutes, participants will provide a saliva sample, complete the battery of behavioural tests and have a short break. The light intervention with the VR headsets will last for 2 hours in total. Throughout this part of the experiment, except when completing the battery of tests, participants will be allowed to listen to pre-selected audiobooks or musical albums.

Finally, at their habitual bedtime, participants will return to the dim room condition for 1 hour. After 30 minutes and at the end of the experiment, they will provide a saliva sample and complete the battery of behavioural tests. They will departure the lab 1 hour after their habitual bedtime.

## Instruments

In this study, we will be using a range of instruments for visual stimulation and measurements. The following table gives the devices, the manufacturer, their purpose, and any certification status.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Device** | **Manufacturer** | **Purpose** | **Certification** | |
| HTC Vive Pro Eye | HTC | Visual stimulation | [Consumer device] | |
| ActTrust2 | Condor (São Pãolo, Brasil) | Measurement of rest-activity cycles and light exposure | IEC60601-1:2006, IEC60601-1-2:2007, IEC60601-1-11:2010 | |
| e-Celsius | BodyCap | Measurement of core body temperature using an ingestible pill | CE |
| OCT2000 | TOPCON | Fundus photographs | CE | |

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

# Participant remuneration

Participants will be remunerated for their time. Upon completion of the screening, they will receive €15. For each session, they will receive €80. If they complete all session, they will receive a bonus of €200.

Risks and benefits

Participants may find adhering to a regular sleep-wake cycle for a period of 5 weeks difficult. Using our screening methods, we identify individuals who will likely not have problems with keeping to a regular sleep-wake cycle.

Participants may find wearing an actigraph tracker for a period of 5 weeks to be intrusive or difficult. The actigraph is no bigger than a wristwatch.

Participants may experience discomfort wearing the VR headsets. We will give participants ample opportunity to wear the VR headsets as a trial prior to committing to the experiment. We will monitor participant comfort through any study visit and will intervene in case participants report feeling uncomfortable.

In sum, we consider the detailed risks to minor given the fundamental biological and biomedical knowledge that can be gained from the study.

# Target criteria

The purpose of this study is to characterise, for the first time, the impact of specific spatial and temporal regimes of light stimulation on neuroendocrine physiology. As the study is geared towards a fundamental characterisation of a biological system, the question of target criteria concerns largely the quality of the protocol. As we have included a dim light condition along with several light-related stimuli,

# 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 in the Max Planck Institute for Biological Cybernetics. 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 study, 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 MPI 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 only 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-lab or online, they are stored on secure, password-protected lab computers and server space accessible only to trained lab 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. Within the framework of cooperation with the bioanalytics facility of the Prevention Centre at the TUM Department of Sports and Health Sciences, the analysis of saliva samples are performed by the laboratory. 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 in the MPI. All participating laboratory physicians and employees are subject to medical confidentiality as stipulated by German law.

## Cooperation and data exchange with other research Institutes

Pseudonymised data will be shared with the Chair of Chronobiology & Health, Technical University of Munich.

## 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 and mood 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 | Data collected on password-protected storage server |
| Ocular and retinal function data, including colour vision and stereo vision status | Data collected on password-protected storage server |
| Ocular coherence tomography (OCT) and fundus images | Data files only drive accessible only to project personnel |
| Saliva samples | Freezers at MPI, shipped to bioanalytics facilities at TUM | 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 |
| Time course of pupil size in mm |
| Time course of eye tracking and gaze data |
| Psychophysical performance and rating data, including visual comfort and mood |
| Time course of core body temperature data |
| Time course of activity and ambient light exposure data measured with actigraph |

# Insurance information

The Max Planck Institute for Biological Cybernetics is insured for public and products liability (Baseler Sachversicherungs-AG, policy number 3184047).

# 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

2022.11.17\_AppendixA\_ActTrust.pdf

2022.11.17\_AppendixA\_BodyCap.pdf

2022.11.17\_AppendixA\_TOPCON.pdf

# Appendix B

2022.11.17\_AppendixB\_AUDIT-de.pdf

2022.11.17\_AppendixB\_AUDIT-en.pdf

2022.11.17\_AppendixB\_LEBA-de.pdf

2022.11.17\_AppendixB\_LEBA-en.pdf

2022.11.17\_AppendixB\_MCTQ-de.pdf

2022.11.17\_AppendixB\_MCTQ-en.pdf

2022.11.17\_AppendixB\_PSQI-de.pdf

2022.11.17\_AppendixB\_PSQI-en.pdf

# References

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