

1    Influence of sex steroid hormones on the  
2    neuroendocrine effects of evening light  
3    exposure in healthy adults

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18 **Supplementary materials**

19 ***Participants compensation strategy***

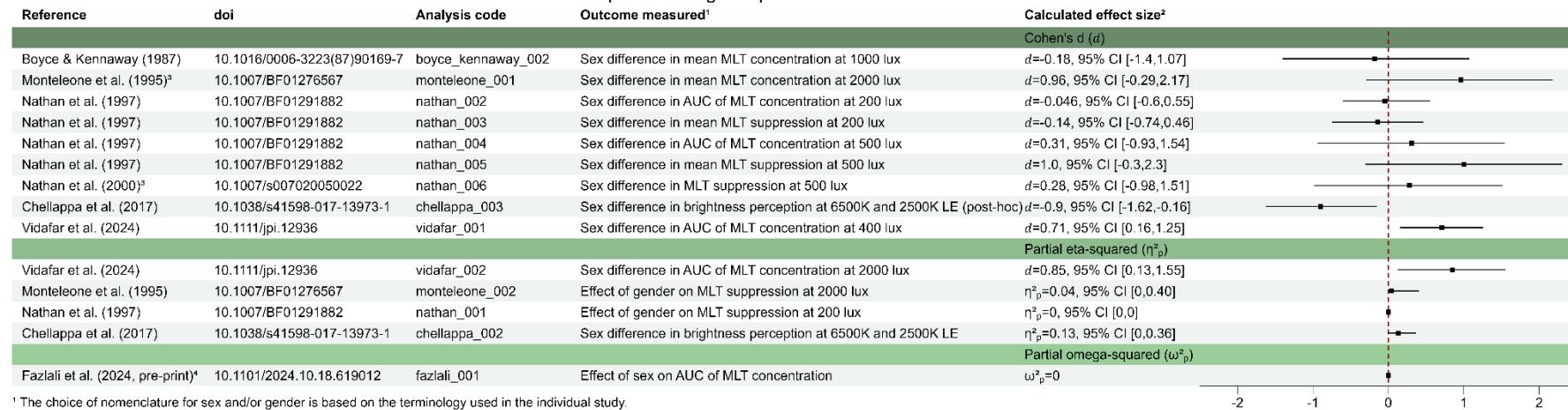
Group	Aspect of the study	Compensation
NC group	Experimental sessions (n=5)	€100/session
	Bonus for flexible scheduling of experimental sessions	€10/session
	LH testing	€1/LH test performed and logged
MCOC group	Experimental sessions (n=5)	€100/session
	Logging pill intake timing	€1/day of logging pill intake
HM group	Experimental sessions (n=5)	€100/session
All groups	Weeks of regular sleep/wake schedule duration and logging (sleep diary)	€10/week
	Logging of mood symptoms	€1/log
	Bonus for completing all experimental sessions and the discharge questionnaire	€100

20 Table S1: Compensation strategy for participation in the study.

21 ***Calculation of effect sizes from existing literature on sex-and  
hormone-related differences in non-visual effects of light***

23 In order to make informed decisions about our study design and sample size, we  
24 performed a detailed overview of effect sizes in existing literature on sex- and hormone-related  
25 differences in non-visual effects of light. We calculated effect size for n=12 studies that we  
26 identified and described using an Open Research Knowledge Graph (ORKG) comparison  
27 (Guidolin & Spitschan, 2024). Calculation of effect sizes and details on the approach taken  
28 are summarised in a reproducible R markdown available at  
29 [https://github.com/tscnlab/GuidolinEtAl\\_PCIRRStage1\\_2025/tree/main/effect\\_sizes](https://github.com/tscnlab/GuidolinEtAl_PCIRRStage1_2025/tree/main/effect_sizes). A  
30 summary of these effect sizes is shown in Figure S1 and S2.

Calculated effect sizes for studies on sex differences in non-visual responses to light exposure



<sup>1</sup> The choice of nomenclature for sex and/or gender is based on the terminology used in the individual study.

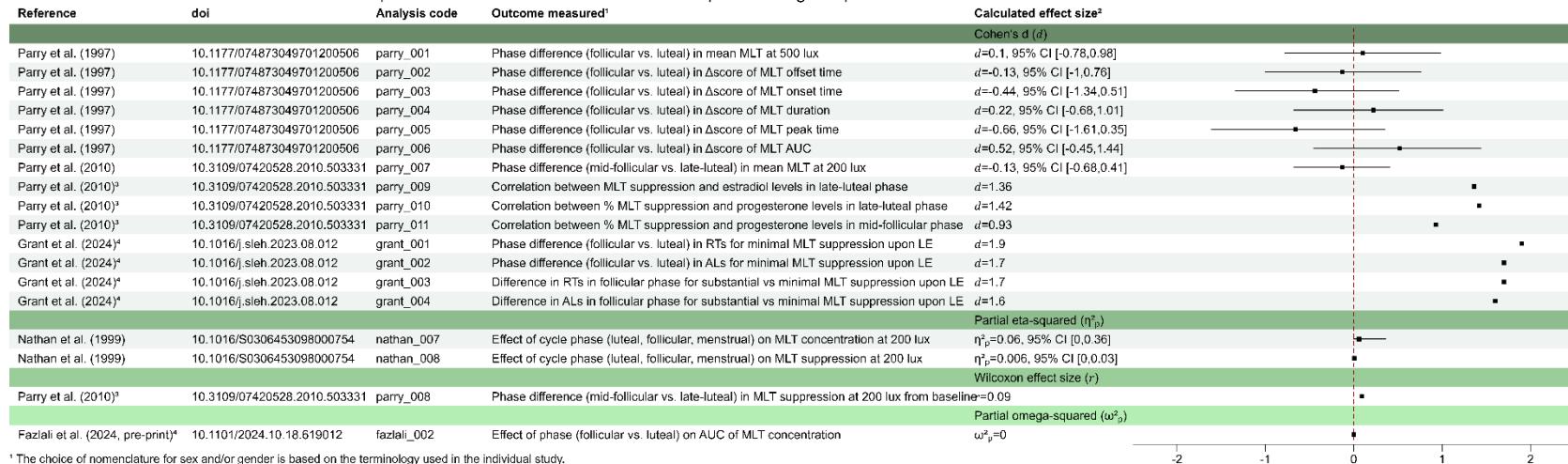
<sup>2</sup> Effect sizes were computed using women/females as Group 1 and men/males as Group 2. Positive effect sizes indicate higher values on the measured outcome for Group 1 compared to Group 2, while negative effect sizes indicate higher values for Group 2.

<sup>a</sup> Data extracted using WebPlotDigitizer.

<sup>b</sup> 95% confidence intervals unavailable (not reported in original study).

Figure S1: Calculated effect sizes for studies on sex differences in non-visual responses to light exposure.

Calculated effect sizes for studies on menstrual phase related differences in non-visual responses to light exposure



<sup>1</sup> The choice of nomenclature for sex and/or gender is based on the terminology used in the individual study.

<sup>2</sup> Effect sizes were computed using women/females as Group 1 and men/males as Group 2. Positive effect sizes indicate higher values on the measured outcome for Group 1 compared to Group 2, while negative effect sizes indicate higher values for Group 2.

<sup>3</sup> 95% confidence intervals unavailable from conversion.

<sup>4</sup> 95% confidence intervals unavailable (not reported in original study).

Figure S2: Calculated effect sizes for studies on menstrual phase related differences in non-visual responses to light exposure.

True positive rate (TPR) for E2 and P4 slope means combinations

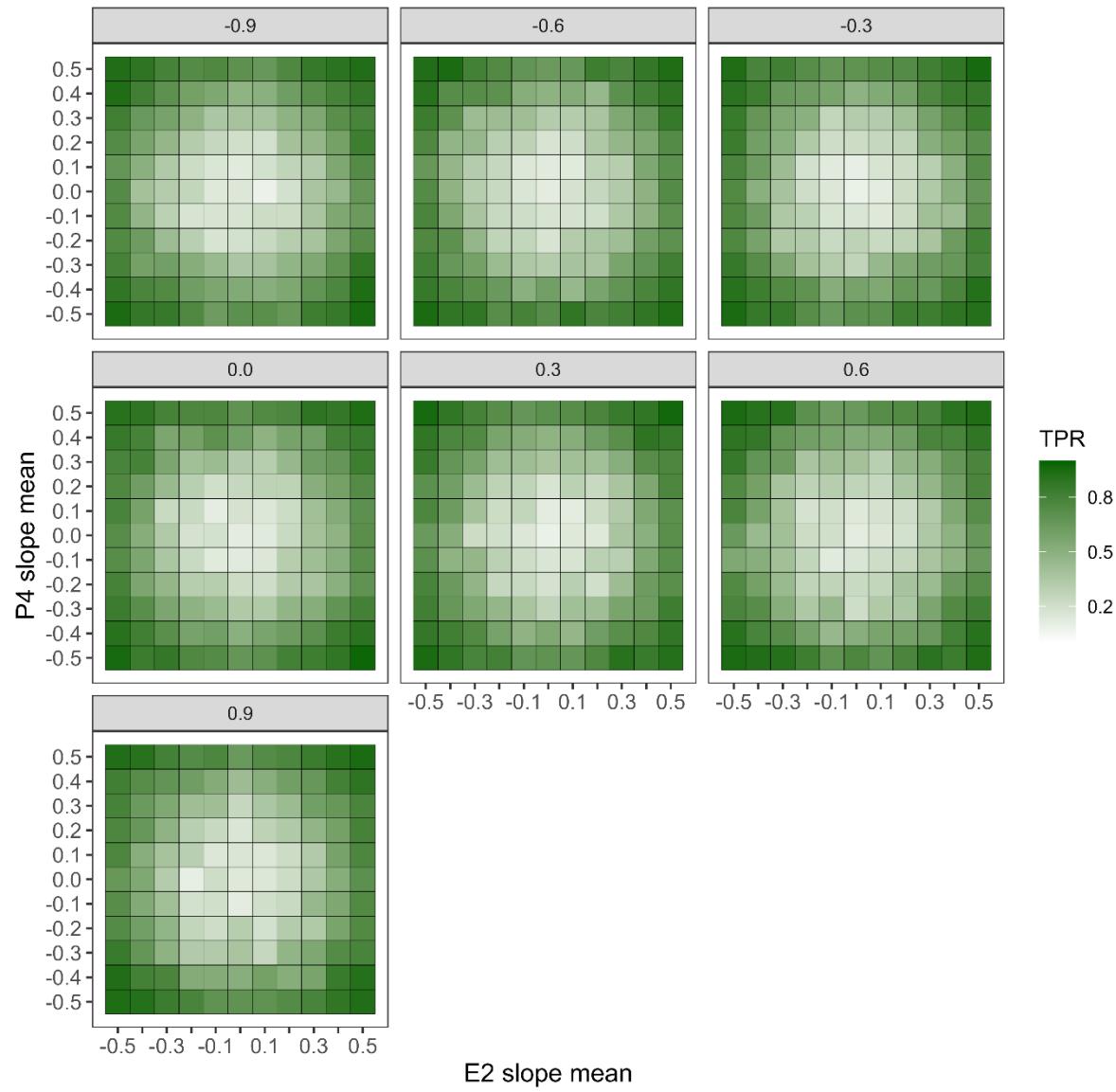


Figure S3: Heatmap of E2 slope mean and P4 mean combinations across intercept mean values. Darker green indicates a higher probability of detecting an effect under the fixed-n BFDA simulation. Each facet represents a different intercept mean value. Each cell in a given facet represents the TPR for the 100 simulations performed drawing from a normal distribution with possible means -0.5 to 0.5 at 0.1 steps and standard deviation 0.2 (for both E2 and P4 slopes), with a given intercept mean value (indicated by the facet label). Intercept values for data simulations are also drawn from a normal distribution with possible means ranging from -0.9 to 0.9 at 0.3 steps and standard deviation 0.2.

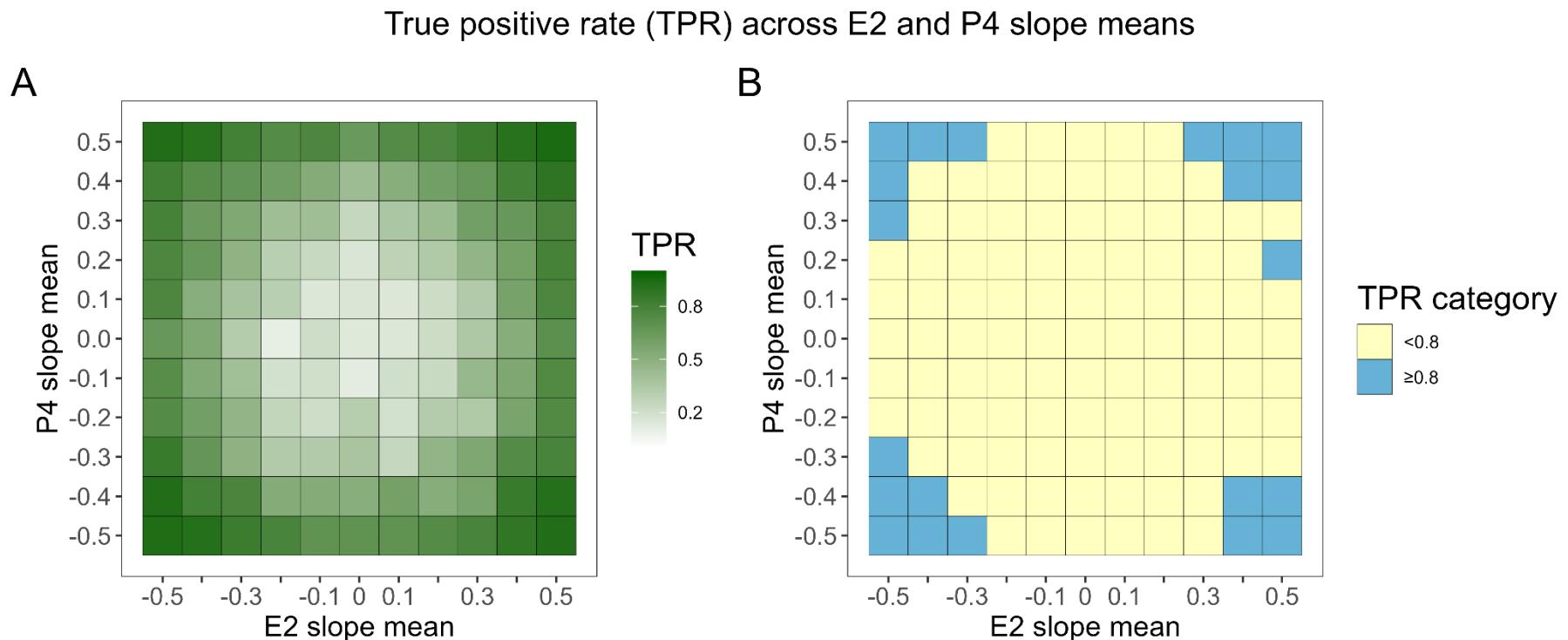


Figure S4: Heatmap of the TPR for each combination of E2 and P4 slope means, aggregated across different intercept mean values. (A) Darker green colours represent high TPR values. (B) Blue cells show E2 slope mean and P4 slope mean combinations yielding high TPR values ( $\geq 0.8$ ). Yellow cells show E2 slope mean and P4 slope mean combinations yielding low TPR values ( $< 0.8$ ).

## ***Stopping principles***

Throughout data collection, various stopping principles will be applied to each participant group.

<b>Group</b>	<b>Aspect</b>	<b>Principle</b>	<b>Assessment method</b>	<b>Adjustment for following experimental sessions</b>	<b>Consequence on already collected data</b>
NC group	Cycle length	Menses onset on cycle 1 occurs $\geq 9$ days prior to predicted menses onset	Self-report of menses onset on mobile app	Participant cannot take place in the study. Participation is possible for future cycles	The dim light condition experimental session data will not be used for analysis. If the participant takes part in the experiment in future cycles, this experimental session has to be repeated.
	Ovulation	Cycle 1 is anovulatory	Self-report on mobile app and picture evidence for no positive result for LH test during cycle 1,	Participant cannot take place in any experimental session during the current cycle, but participation is possible for future cycles	The already collected data for the dim light condition and the peri-ovulatory phase will not be included in the analysis. If the participant takes part in the experiment in future cycles, both

			even if E2 positive test is obtained		experimental sessions have to be repeated.
	Cycle length	Luteal phase of cycle 1 ≤9 days (day after positive LH test to day before subsequent menses onset)	Self-report of menses onset on mobile app	Participant cannot continue participation in the study, but participation is possible for future cycles	The already collected data for the dim light condition can be kept and, if the participant decides to take part in future cycles, it does not have to be repeated. Peri-ovulatory and mid-luteal sessions would have to be rescheduled
	Ovulation	Cycle 2 is anovulatory	Self-report on mobile app and picture evidence for no positive result for LH-test during cycle 2, even if estradiol peak detected in the days before	N/A, no future experimental sessions are planned	No influence on peri-ovulatory, mid-luteal, and perimenstrual session. Data from the mid-follicular phase has to be re-collected. If this is not possible, it will not be included in the analysis

	Cycle length	Luteal phase of cycle 2 ≤9 days (day after positive LH test to day before subsequent menses onset)	Self-report of menses onset on mobile app	N/A, no future experimental sessions are planned	No influence on periovulatory, mid-luteal, and perimenstrual session. Data from the mid-follicular phase has to be re-collected. If this is not possible, it will not be included in the analysis
<b>MCOC group</b>	Pill intake	Took the pill at the wrong time, i.e. not after wake-up	Lack of timely self-report on mobile app, or email communication to researcher	Participant is asked to take the pill as soon as possible. If this is the day of an experimental session, participation is possible if the oral contraceptive is taken at least 10 hours prior to HBT	No consequence, since not influenced
<b>All participants</b>	Circadian stabilisation	1x deviation from target bed or wake time > ±30 minutes in the three days prior to an	Sleep diary and visual check of actigraphy data	Participant cannot participate in future experimental sessions	Data from other experimental sessions is kept and included in the analysis

		experimental session			
	Alcohol intake	Alcohol intake on experimental day	Breathalyzer ACE results>0.05	Participant cannot participate in the current experimental session	No consequence. Experimental session will have to be re-scheduled
	Drug use	Drug consumption on experimental day	Drug-Screen Multi 5 positive (any positive test on the multi-panel)	Participant cannot participate in the current experimental session	No consequence. Experimental session will have to be re-scheduled
	Ability to follow instructions	Participant is not able to adhere to the protocol	In-person interaction with the experimenter (experimenter judgement)	Participant is excluded from the study	No further experimental sessions will be performed

Table S2. Overview of the stopping criteria for this study, and consequences for already collected data and to-be-collected data.

## Scheduling principles: NC group participants

March						
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
24	25	26	27	28	1	2
3	4	5	6	7	8	9
				In-person screening		
10	11	12	13	14	15	16
			Dim-light control session			
17	18	19	20	21	22	23
					Menses onset (cycle 1)	
24	25	26	27	28	29	30
					Negative E2 and LH test	Negative E2 and LH test
31	1					
Negative E2 and LH test						

April						
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
31	1	2	3	4	5	6
	Positive E2 test	(Estimated positive LH test day) Positive E2 test	Peri-ovulatory session Positive E2 test	Positive E2 test	Positive LH test	
7	8	9	10	11	12	13
				+6	+7	+8
14	15	16	17	18	19	20
Mid-luteal session +9	+10	Estimated menses onset +11	+12	+13	+14	Menses onset (cycle 2) +15
21	22	23	24	25	26	27
Peri-menstrual session +16	+3	+4	Mid-follicular session +5	+6	+7	+8
28	29	30	1	2	3	4
5	6					

Figure S5: Example for scheduling a participant of the NC group based on their at-home E2 and LH test results and menses onset. Cells with red contour represent the already planned experimental sessions for the MCCOC and HM groups, always falling on Mondays and Thursdays. At screening (i.e. based on data for last six cycles), the participant represented here has an average cycle length of 26 days, and the shortest cycle length is also 26 days. Given the in-person screening on Friday, 7 March, the dim light control session for this NC participant will occur on Thursday, 13 March, following at least five days of circadian stabilisation. Once the participant reports menses onset for cycle 1 (Saturday, 22 March), the day when they should start E2 and LH testing can be calculated based on their shortest cycle length (i.e. menses onset based on shortest cycle length = Wednesday, 16 April). Subtracting 14 days from this date leads to Wednesday, 2 April, and the participants should start testing four days prior this date, meaning Saturday, 29 March). The estimated positive LH test day can also be estimated based on average cycle length of the last six months. Here, it would fall on Wednesday, 2 April. Since

an experimental session is planned on Thursday, 3 April, the participant is provisionally scheduled for this date. This experimental session is then confirmed because the participant obtains an E2 positive test on Tuesday, 1 April. The participant then continues testing until they obtain a positive LH test result (here, Saturday, 5 April). From this day, the mid-luteal (red coloured cells) and peri-menstrual (light blue coloured cells) experimental sessions can be scheduled +6 to +10 and +12 to +16 days from positive LH test day (positive LH test day = 0). In this case, the mid-luteal phase session would fall on Monday, 14 April and the peri-menstrual phase session could fall on Thursday, 17 April or Monday, 21 April. The participant reports menses onset for cycle 2 on Sunday, 20 April, and the mid-follicular phase (lilac coloured cells) session can then be scheduled in the time window +4 to +8 from menses onset (which corresponds to day +1). Here, it would take place on Thursday, 24 April. Note that the cycle length for cycle 1 is longer than estimated (29 days rather than 26 based on average). However, our scheduling method still captures all phases as planned, and the peri-ovulatory session takes place prior to the positive LH test, ensuring high and rising levels of estradiol are captured.

## ***Light-level characteristics for light stimuli delivery through the VR headsets***

Stimulus properties for dim light condition (<0.01 lx)						
VR	Photopic illuminance [lux] (mean±1SD)		Melanopic EDI [lux] (mean±1SD)		Intra-VR headset averages (mean±1SD)	
	Left eye	Right eye	Left eye	Right eye	Photopic illuminance	Melanopic EDI
1	0.002±0.003	0.01±0.013	0.008±0.013	0.008±0.008	0.006±0.008	0.008±0.011
2	0.004±0.004	0.011±0.009	0.004±0.007	0.009±0.011	0.007±0.007	0.007±0.009
3	0.007±0.009	0.003±0.003	0.005±0.006	0.01±0.014	0.005±0.006	0.007±0.01
4	0.011±0.013	0.004±0.005	0.014±0.019	0.003±0.003	0.008±0.009	0.009±0.011
5	0.012±0.022	0.012±0.013	0.006±0.011	0.008±0.006	0.012±0.018	0.007±0.009
Inter-VR averages (mean±1SD)	0.007±0.01	0.008±0.009	0.008±0.011	0.008±0.008		

Stimulus properties for bright condition (~90 lx)						
VR	Photopic illuminance [lux] (mean±1SD)		Melanopic EDI [lux] (mean±1SD)		Intra-VR headset averages (mean±1SD)	
	Left eye	Right eye	Left eye	Right eye	Photopic illuminance	Melanopic EDI
1	103.68±0.46	92.4±1.11	103.48±0.36	97.2±0.8	98.04±0.78	100.34±0.58
2	96.12±0.08	92.98±0.08	95.81±0.04	92.75±0.08	94.55±0.08	94.28±0.06
3	91.81±0.03	79.39±0.3	89.04±0.04	81.33±0.21	85.6±0.16	85.18±0.13
4	89.07±1.05	88.69±0.14	89.32±0.72	91.41±0.09	88.88±0.6	90.36±0.41
5	92.77±0.07	76.21±0.17	93.28±0.07	75.82±0.24	84.49±0.12	84.55±0.16
Inter-VR averages (mean±1SD)	94.69±0.34	85.93±0.36	94.18±0.25	87.7±0.28		

Table S3: Calibration results for photopic illuminance and melanopic EDI delivery through the five available VR headsets. Each cell of the “Left eye” and “Right eye” columns represents the mean±1SD of five repeats at input intensity 0 (top panel, dim light condition) and at input intensity 0.5 (bottom panel, bright light condition). Intra-VR and inter-VR averages (mean±1SD) are shown.

**Table 1. SAGER guidelines checklist: Studies with human participants**

Section / topic	Item number	Checklist item	Reported on page number
<b>General</b>			
	1	The terms sex/gender used appropriately	p.8
<b>Title</b>			
	2	Title specifies the sex/gender of participants if only one included	N/A, both included
<b>Abstract</b>			
	3a	Abstract specifies the sex/gender of participants if only one included	N/A, both included
	3b	Study population described with sex/gender breakdown*	Will report after data collection
<b>Introduction</b>			
	4a	If relevant, previous studies that show presence or lack of sex/gender differences or similarities are cited	pp. 5-6
	4b	Mention of whether sex/gender might be an important variant and if differences might be expected	pp. 7-8
	4c	The demographics of the study population with regard to sex/gender (eg, disease prevalence among male/female study participants) are outlined*	Will report after data collection
<b>Methods</b>			
	5a	Method of definition of sex/gender (eg, self-report, genetic testing)	pp. 9-11
	5b	Description of how sex/gender was considered in the design, whether authors ensured adequate representation of male and female study participants, justification of the reasons for any exclusion of male or female participants, or explanation if not considered. Justification of other sex/gender-specific interventions of study designs (eg, mandating contraception for women).* Explicit reporting of the scientific rationale for	p. 7

		contraception requirements and exclusions for pregnancy and lactation should be required*	
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## Results

	6a	Study population description with complete gender/sex breakdown for all categories considered*	Will report after data collection
	6b	Where appropriate, data presented disaggregated by sex/gender, and sex/gender differences and similarities are described	Will report after data collection
	6c	Sex- and gender-based analyses reported regardless of outcome (in main paper if pre-specified; otherwise in appendix)*	Will report after data collection
	6d	For clinical trials, adverse event data disaggregated by sex/gender (in main paper if pre-specified; otherwise in appendix)*	Will report after data collection
	6e	Patient-reported outcome data disaggregated by sex/gender (in main paper if pre-specified; otherwise in appendix)*	Will report after data collection
	6f	For epidemiological studies, the effects of other exposures on health problems examined for all genders and analysed critically from a gender perspective	Will report after data collection
	6g	Table 1 includes separate rows for male sex/gender, female sex/gender and other categories if collected*	Will report after data collection

## Discussion

	7a	Potential implications of sex/gender on the study results and analyses, including the extent to which the findings can be generalized to all sexes/genders in a population	Will report after data collection
	7b	If a sex/gender analysis not done, a rationale is given and implications of the lack of such analysis on the interpretation of the results are discussed	Will report after data collection

Adapted from SAGER guidelines. Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. Research Integrity and Peer Review 1, Article number: 2 (2016) <https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6>.

\* These points extend beyond the original SAGER table

# ENLIGHT Checklist

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## General Information

**Author names:** Carolina Guidolin and Manuel Spitschan

**Title of manuscript:** Influence of sex steroid hormones on the neuroendocrine effects of light at night

**Date:** 12 June 2025

## A. Study Characteristics

### A.1. Protocol-level characteristics

	Location (page, figure, table number)	Not available	Not applicable
Description of experimental setting	pp. 23-25	<input type="checkbox"/>	
Timeline of experiment (including timing and duration of light)	pp. 23-25, Figure 2	<input type="checkbox"/>	
Pre-laboratory sleep-wake/rest-activity behaviour	p. 23-25	<input type="checkbox"/>	<input type="checkbox"/>
Pre-laboratory light exposure	pp. 31-32	<input type="checkbox"/>	<input type="checkbox"/>
Immediate prior light exposure (in laboratory)	pp. 23-25	<input type="checkbox"/>	<input type="checkbox"/>

### A.2. Measurement-level characteristics

Measurement plane (e.g., horizontal or vertical)	pp. 25-26	<input type="checkbox"/>	
Measurement viewpoint and location	pp. 25-26	<input type="checkbox"/>	
Type, make and manufacturer of the measurement instrument	pp. 25-26	<input type="checkbox"/>	
Calibration status of the instrument	pp. 25-26	<input type="checkbox"/>	<input type="checkbox"/>

### A.3. Participant-level characteristics

Ocular health and functioning	pp. 10-16, Tables 1-4	<input type="checkbox"/>	
Pupil size and/or dilation	pp. 23-25	<input type="checkbox"/>	<input type="checkbox"/>
Relative time (e.g. to circadian phase or sleep)	pp. 23-25	<input type="checkbox"/>	<input type="checkbox"/>

## B. Light characteristics

### B.1. Light source type(s). Please select all that are relevant.

Room illumination (overhead or other)	Emissive surfaces including displays (incl. light therapy devices)	Wearable light emitting glasses	Ganzfeld exposure	Other: <b>Virtual reality head-mounted display</b>
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Polychromatic light

Monochromatic or narrowband light

	<b>Location (page, figure, table number)</b>	<b>Not available</b>	<b>Not applicable</b>
Type, make and manufacturer of the light source	pp. 23-25	<input type="checkbox"/>	
Use of wearable filtering apparatus (e.g., blue-blocking glasses)		<input type="checkbox"/>	<input checked="" type="checkbox"/>

### B.2. Light level characteristics

ILLuminance (lux) and/or luminance (cd/m <sup>2</sup> )	pp. 25-26	<input type="checkbox"/>	
Spectral irradiance and/or radiance distribution	Supplementary materials (digital)	<input type="checkbox"/>	<input type="checkbox"/>
α-opic irradiance and/or radiance (including melanopic)	pp. 25-26	<input type="checkbox"/>	<input type="checkbox"/>
α-opic equivalent daylight illuminance and/or luminance (EDI/EDL, including melanopic)	pp. 25-26	<input type="checkbox"/>	<input type="checkbox"/>

NOTE: Luminance and radiance metrics (as opposed to illuminance and irradiance) are mainly relevant for emissive surfaces.

### B.3. Colour characteristics

Peak wavelength and bandwidth		<input type="checkbox"/>	<input checked="" type="checkbox"/>
Colour appearance quantities (any)		<input type="checkbox"/>	<input checked="" type="checkbox"/>
Colour rendering metrics (any)		<input type="checkbox"/>	<input checked="" type="checkbox"/>

NOTE: Peak wavelength and bandwidth are most relevant for monochromatic or narrowband light sources.

### B.4. Temporal and spatial characteristics

Location of stimulus and viewing distance	pp. 25-26	<input type="checkbox"/>	
Temporal pattern (including flash frequency and waveform)	None	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Relative or absolute size of the stimulus	pp. 25-26	<input type="checkbox"/>	<input type="checkbox"/>