Check for updates

*Journal of Pineal Research*

WILEY

Journal of Pineal Research
Molecular, Biological, Physiological and Clinical Aspects of Melatonin

ORIGINAL ARTICLE OPEN ACCESS

# Impact of Human Melanopsin Gene (*OPN4*) Polymorphisms (I394T, P10L) on Nocturnal Melatonin and Daily Rhythms in Young Adults

Jesús Vicente-Martínez[1,2,3] | María Ángeles Bonmatí-Carrión[2,4] | Ana María López-Parra[5] | Juan Antonio Madrid[1,2,3] | Pedro Francisco Almaida-Pagán[1,2,3] | María Angeles Rol[1,2,3]

[1]Chronobiology and Sleep Laboratory, University of Murcia, Mare Nostrum Campus, IUIE, Murcia, Spain | [2]Biomedical Research Institute of Murcia Pascual Parrilla–IMIB, Murcia, Spain | [3]Ciber Fragilidad y Envejecimiento Saludable (CIBERFES), Instituto de Salud Carlos III, Madrid, Spain | [4]Department of Human Anatomy and Psychobiology, Faculty of Medicine, University of Murcia, Mare Nostrum Campus, Murcia, Spain | [5]Laboratory of Forensic and Population Genetics, Legal Medicine, Psychiatry and Pathology Department, Faculty of Medicine, Complutense University of Madrid, Madrid, Spain

**Correspondence:** María Ángeles Bonmatí-Carrión (mabonmati@um.es)

**Received:** 20 April 2026 | **Revised:** 27 July 2026 | **Accepted:** 30 July 2026

**Funding:** Ministry of Economy and Competitiveness and the Instituto de Salud Carlos III, Grant/Award Number: CB16/10/00239; MCIN, Grant/Award Number: PID2022-136577OB-I00; Fundación Séneca, Grant/Award Number: 21610/FPI/21

**Keywords:** aging | circadian rhythm | melanopsin | melatonin | single nucleotide polymorphism | skin temperature | sleep

## ABSTRACT

The present study investigates the impact of two missense SNPs in the human melanopsin gene *OPN4* -I394T (rs1079610) and P10L (rs2675703)- on non-image-forming (NIF) physiological and behavioural outputs in healthy young adults under real-world conditions. Twenty-five healthy university students were genotyped and analysed under a C-allele dominant model for I394T and a T-allele dominant model for P10L. Over 8 days, participants underwent ambulatory monitoring of wrist skin temperature (WT), sleep, activity and light exposure, together with salivary melatonin assessment under five home-based lighting/time conditions and pupillary light reflex testing. I394T-C carriers exhibited a phase delay in habitual sleep and WT rhythms, reduced WT amplitude, greater internal desynchronization, and lower nocturnal melatonin concentrations under dim light compared to the TT group. Although behavioural and peripheral rhythms were delayed, circadian phase (DLMO) remained preserved, resulting in an increased phase angle of entrainment. On free days, they also showed greater melatonin suppression despite similar light exposure. In contrast, the P10L-T allele was not associated with significant differences in ambulatory, hormonal, or pupillary outcomes. Overall, the I394T variant, but not P10L, significantly associates with NIF physiology in young adults, likely by affecting the coupling between environmental light and downstream circadian outputs, independently of circadian phase. These findings also suggest that I394T-C carriers exhibit features of the circadian phenotype commonly observed in older cohorts, including reduced rhythm robustness and lower melatonin levels, and support a role for this variant in interindividual differences in light sensitivity and circadian function.

## 1 | Introduction

The circadian system orchestrates the 24-h temporal organisation of physiology and behaviour and is entrained primarily by light [1]. In mammals, its central pacemaker is located in the suprachiasmatic nuclei (SCN) of the hypothalamus [2, 3]. This synchronisation depends critically on the non-image-forming (NIF) visual pathway, mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs), which act as photoreceptors alongside rods and cones [4–6]. These phototransduction

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2026 The Author(s). *Journal of Pineal Research* published by John Wiley & Sons Ltd.

**TABLE 1** | Observed (left) and expected (right) contingency between SNPs I394T and P10L ($N = 25$).

| Observed | | I394T | | | Total | Expected | | I394T | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TT | TC | CC | | | | TT | TC | CC | |
| P10L | CC | 4 | 10 | 1 | 15 | P10L | CC | 6 | 8 | 1 | 15 |
| | CT | 6 | 3 | 0 | 9 | | CT | 4 | 5 | 0 | 9 |
| | TT | 0 | 1 | 0 | 1 | | TT | 0 | 1 | 0 | 1 |
| Total | | 10 | 14 | 1 | 25 | Total | | 10 | 14 | 1 | 25 |

signals are essential for circadian photoentrainment [7, 8], pupillary light responses (PLR) [9, 10], light-induced melatonin suppression [11–14], and the regulation of sleep and peripheral temperature rhythms [14–16].

The core photopigment in ipRGCs is melanopsin, a blue light-sensitive opsin encoded by the *OPN4* gene [4]. Because of its central role in the NIF pathway, variations in *OPN4* has been proposed to contribute to interindividual differences in light sensitivity and downstream circadian function [17, 18]. Among its variants, two missense SNPs -Pro10Leu (P10L, rs2675703) and Ile394Thr (I394T, rs1079610)- have attracted particular interest. The P10L-T allele has been associated with chronic insomnia [19], seasonal affective disorder [20], and later sleep onset and chronotype [17], whereas the I394T C allele has been linked to enhanced PLR [21, 22] and later sleep/wake timing [23]. However, although population studies support associations with altered NIF behaviours, the direct functional consequences of these melanopsin variants remain unclear, as *in vitro* and *in vivo* evidence has been conflicting [18, 24].

Assessing the influence of melanopsin variants on NIF responses in real-life settings remains challenging because of the diversity and complexity of these outputs. Melatonin suppression may provide a useful index of melanopsin function, given their close functional and anatomical association [25], and although individuals show > 50-fold variability in light-induced melatonin suppression [26], no study had yet examined the effect of melanopsin variants on melatonin suppression and melatonin secretion under dim light conditions. Likewise, PLR represents a non-invasive assay of melanopsin-mediated phototransduction because it is driven predominantly by ipRGCs [10], but its interpretation in daily life is complicated by the fact that light exposure varies widely in intensity, spectrum, and timing, making it difficult to isolate melanopsin-driven responses from those of rods and cones [27]. In this context, circadian ambulatory monitoring of activity, peripheral temperature, and light exposure provides a valuable complementary approach for assessing daily NIF outputs under real-world conditions [16, 28]. Wrist-worn devices, in particular, offer a practical and scalable method, with advantages including low participant burden, continuous long-term recording, high compliance [29], and the ability to capture individual variability in real-world environments across occupational, social, and seasonal contexts [30–32].

Together, these considerations highlight the need for integrative approaches linking genetic variation, physiological outputs, and real-world behaviour. Identifying the functional consequences of *OPN4* variants may improve understanding of the mechanisms underlying circadian vulnerability, sleep disturbances, and light-related disorders. Accordingly, this study examined the impact of the *OPN4* P10L and I394T variants in healthy young adults by combining genetic analysis, melatonin profiling, continuous ambulatory monitoring of wrist skin temperature, sleep and light exposure, and pupillary light responses, thereby helping to bridge the gap between genetic association and mechanistic consequence (Table 1).

## 2 | Materials and Methods

### 2.1 | Participants

Twenty-five healthy Spanish university students ($22.4 \pm 1.6$ years, mean ± SD; five men) were recruited. All were in good health, reported no medical or psychiatric disorders, and did not use medication affecting circadian rhythms, sleep, or melatonin production. None reported shift work or showed colour blindness on the Ishihara test. All participants provided written informed consent in accordance with the Helsinki Declaration and received compensation. The study was approved by the Ethics Committee of the University of Murcia (CEI 2108/2018) and conducted in accordance with relevant guidelines and regulations.

### 2.2 | Genotyping of I394T and P10L SNPs

Saliva samples were collected for genotyping of two *OPN4* coding SNPs: rs1079610 (I394T) and rs2675703 (P10L). DNA was extracted using Chelex®100. Target regions were amplified via PCR and then purified to remove residual reagents [33]. Sequencing was completed through Sanger sequencing on a 3500 Genetic Analyzer. A detailed description of the procedures can be found in Appendix A.

### 2.3 | Ambulatory Monitoring and Sleep Inference

Participants were monitored for eight consecutive days, starting on Monday morning (Figure 1A), and were instructed to maintain their habitual lifestyle, except on the three evenings of saliva collection (Tuesday, Thursday, and Saturday), when they were asked to remain at home from at least 30 min before the first sample. Each participant wore a wrist-worn ambulatory monitoring device (Kronowise®3.0, Kronohealth SL, Murcia, Spain) on the non-dominant hand, which continuously recorded wrist skin temperature (WT), triaxial acceleration,



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

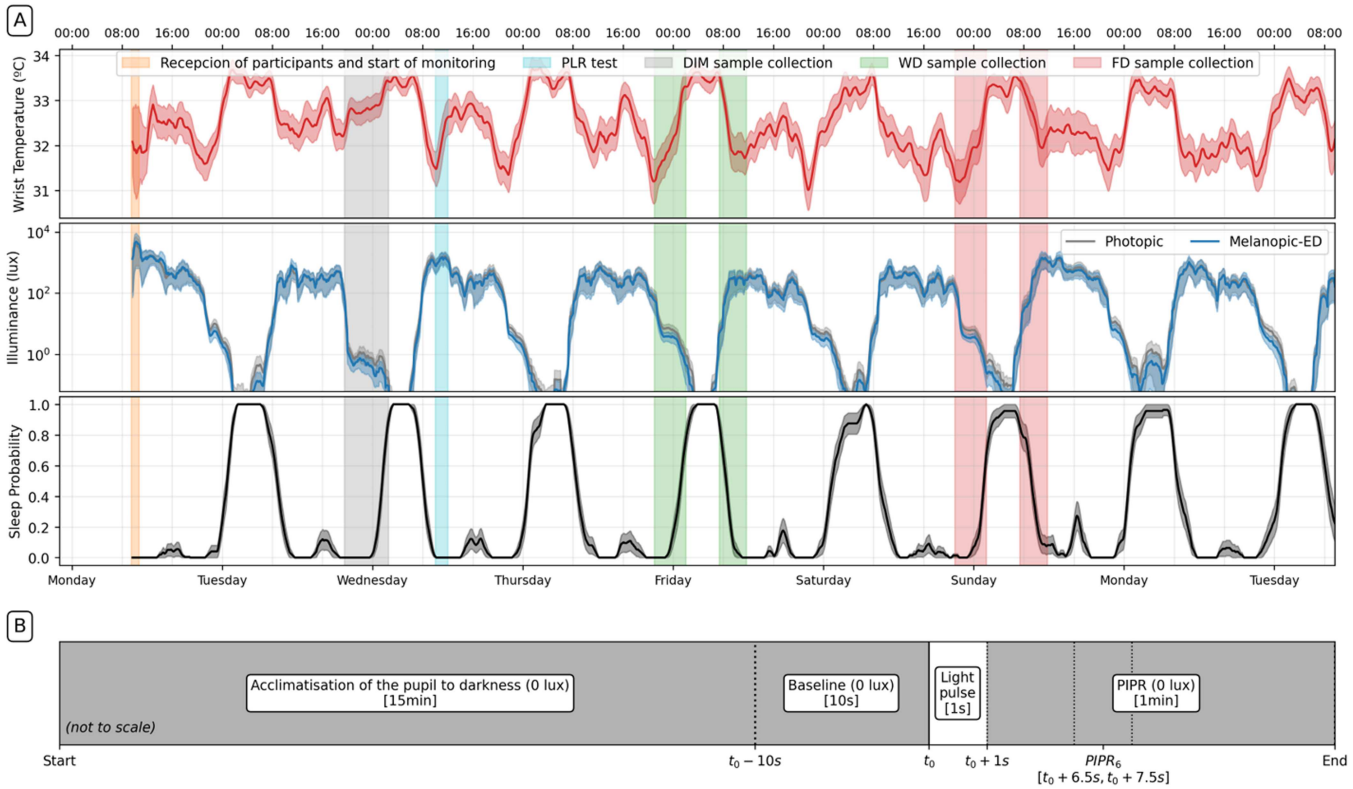


**FIGURE 1** | (A) Average ($N = 25$) ambulatory monitoring variables throughout the protocol week and (B) experimental design for pupil light reflex (PLR) assessment. (A) Variables recorded included wrist temperature (top), photopic and melanopic equivalent daylight illuminances (middle) and sleep probability (bottom). Sleep/wake periods were inferred from acceleration and wrist position recordings (not plotted), and sleep probability was calculated from them. Monitoring started on Monday morning and saliva sampling occurred in a control evening (DIM, grey), a weekday evening and the following morning (WD, green) and a free day evening and the following morning (FD, red). PLR test was performed on Wednesday morning. Note compliance with dim light conditions (< 1 lux m-EDI) on Tuesday evening (control day). (B) The PLR protocol consisted of 15 min of acclimatisation of the pupil to darkness followed by the recording of the pupil by an infrared camera, including: baseline (last 10 s before the light pulse), light pulse (1 s) and post-illumination pupil response (1 min after the light pulse). The $PIPR_6$ was calculated by averaging the pupil from 5.5 to 6.5 s after the light pulse end.

wrist position, and exposure to photopic illuminance and melanopic equivalent daylight illuminance (m-EDI) [34, 35]. Sleep and wake periods were manually identified by a trained researcher from acceleration and wrist position data. For this study, only sleep/wake cycle, WT, and m-EDI were included in the analyses.

Sleep, WT, and m-EDI were characterised using their weekly mean waveform, with phase markers and amplitude assessed by non-parametric analysis [36]. Phase markers included sleep midpoint, WT TM5 (midpoint of the five consecutive hours with the highest wrist temperature, typically at night), and m-EDI TM10 (midpoint of the ten consecutive hours with the highest m-EDI values, typically during daytime). Amplitude markers included the absolute amplitudes of WT and m-EDI (difference between the most and least active periods) and sleep duration.

## 2.4 | Desynchronization Between the Ambulatory Variables

Temporal desynchronization between ambulatory variables was assessed by pairwise cross-correlation analyses of the mean daily waveforms of sleep probability, WT, and m-EDI for each participant, performed separately for weekdays and free days. Desynchronization indices were calculated by normalising the absolute cross-correlation lag to its maximum possible value (12 h, representing perfectly opposed waveforms). To ensure consistency, indices involving m-EDI were inverted to account for its antiphasic relationship with WT and sleep, since m-EDI typically peaks during the daytime, whereas WT and sleep peak during the nighttime. A similar approach based on non-parametric phase markers has been used previously [37, 38].

## 2.5 | Saliva Sampling for Melatonin Assessment

Salivary melatonin was assessed at home under five experimental conditions: a dim-light control evening (DIM, Tuesday), a weekday evening under habitual light (WDE, Thursday), a free day evening under habitual light (FDE, Saturday), a weekday morning (WDM, Friday), and a free day morning (FDM, Sunday), following the guidelines in [39]. Participants received all materials required for collection, including labelled vials, thermal bags, and sample logs, and were instructed to remain seated, avoid eating or drinking for at least 15 min before each sample, and store samples refrigerated (~ 4°C) until returning them to the laboratory.



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

**TABLE 2** | Coefficients, standard errors, *p*-values of the Wald tests and confidence intervals of the parameters of the linear mixed-effect model fittings for each variable and marker, including the I394T SNP as predictor. A total of sixfittings were performed. Please note that phase markers' intercepts are expressed in h:mm format (since they represent a time point), whereas delays or advances caused by genotype and day are expressed in decimal hours (since they represent a time interval).

| **Wrist temperature** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **TM5** | | | | | **Amplitude (°C)** | | | | |
| | **Coeff.** | **Stand. Error** | ***p* value** | **[0.025** | **0.975]** | **Coeff.** | **Stand. Error** | ***p* value** | **[0.025** | **0.975]** |
| Intercept | 3:34 | 0:59 | 0.0004 | 1:37 | 5:31 | 1.41 | 0.21 | < 0.0001 | 0.99 | 1.82 |
| Gen. I394T [TC + CC] | 3.49 | 1.12 | 0.0019 | 1.29 | 5.70 | −0.65 | 0.24 | 0.0075 | −1.13 | −0.18 |
| Day [FD] | 0.31 | 1.07 | 0.7682 | −1.78 | 2.40 | −0.13 | 0.20 | 0.5225 | −0.52 | 0.27 |
| **Sleep** | | | | | | | | | | |
| | **Midpoint** | | | | | **Duration (h)** | | | | |
| | **Coeff.** | **Stand. Error** | ***p* value** | **[0.025** | **0.975]** | **Coeff.** | **Stand. Error** | ***p* value** | **[0.025** | **0.975]** |
| Intercept | 4:14 | 0:17 | < 0.0001 | 3:41 | 4:47 | 7.57 | 0.27 | < 0.0001 | 7.05 | 8.10 |
| Gen. I394T [TC + CC] | 0.85 | 0.34 | 0.0132 | 0.18 | 1.53 | −0.20 | 0.31 | 0.5218 | −0.82 | 0.41 |
| Day [FD] | 0.82 | 0.18 | < 0.0001 | 0.46 | 1.18 | −0.09 | 0.24 | 0.7170 | −0.56 | 0.38 |
| **m-ED Illuminance** | | | | | | | | | | |
| | **TM10** | | | | | **Amplitude (melanopic lux)** | | | | |
| | **Coeff.** | **Stand. Error** | ***p* value** | **[0.025** | **0.975]** | **Coeff.** | **Stand. Error** | ***p* value** | **[0.025** | **0.975]** |
| Intercept | 14:08 | 0:19 | < 0.0001 | 13:31 | 14:44 | 165.92 | 40.62 | < 0.0001 | 86.31 | 245.52 |
| Gen. I394T [TC + CC] | 0.33 | 0.38 | 0.3898 | −0.42 | 1.08 | −2.99 | 45.69 | 0.9479 | −92.54 | 86.57 |
| Day [FD] | 0.86 | 0.23 | 0.0002 | 0.40 | 1.32 | 52.87 | 44.78 | 0.2378 | −34.90 | 140.64 |

On all evening conditions (DIM, WDE, FDE), saliva was collected at 30-min intervals. On DIM, sampling was performed from 19:30 until 1 h after habitual bedtime, following a modified protocol from [28]. Participants were instructed to remain seated under dim light and avoid light-emitting devices. Compliance with dim light conditions was verified through ambulatory recordings of photopic and melanopic illuminance from the wrist-worn device. On WDE and FDE, sampling was conducted from 2 h before habitual bedtime until bedtime, also at 30-min intervals, while participants maintained their habitual indoor lifestyle and usual light exposure. On WDM and FDM, saliva was collected immediately upon waking. No light restrictions were imposed during WDE, FDE, WDM, or FDM, including the use of light-emitting electronic devices.

## 2.6 | Melatonin Quantification and DLMO Calculation

Salivary melatonin concentrations were quantified in duplicate using competitive ELISA (Melatonin direct Saliva ELISA, IBL International GmbH – Tecan Group, Hamburg, Germany), with a detection limit of 1.6 pg/ml. Samples were stored at −20°C for at least 48 h before quantification, according to the assay protocol.

The average intra-assay CVs were 14.12% for low-level pools (2.407 ± 0.334 pg/mL) and 16.62% for high-level pools (23.632 ± 4.126 pg/mL). The inter-assay CVs were 20.89% for low-level pools (2.407 ± 0.503 pg/mL) and 20.85% for high-level pools (23.632 ± 4.928 pg/mL).

Dim Light Melatonin Onset (DLMO) was calculated from the DIM samples using the dynamic (2 SD) method [40], with the threshold defined as the mean + 2 SD of the first three baseline samples. Each DLMO was then visually checked against its full melatonin profile by two trained researchers.

## 2.7 | Pupillary Light Reflex

The pupillary light reflex (PLR) was assessed in all participants (Figure 1B) on Wednesday morning, at similar clock times to minimise circadian effects on pupil responses [41, 42], following published pupillography standards [43]. A detailed description of the procedures can be found in Appendix B.

## 2.8 | Statistical Analysis

Ambulatory monitoring data (waveforms, phase, duration, and amplitude markers) were averaged by I394T genotype (TT vs TC + CC) and P10L genotype (CC vs CT + TT) over the entire 8-day period, for weekdays (WD; Sunday 16:00:00 - Friday 15:59:59), free days (FD; 16:00:00 - Sunday 15:59:59), and day-to-day. Average melatonin levels and corresponding m-EDI light exposures were calculated from DLMO onwards, excluding baseline samples. Melatonin suppression during WDE and FDE evenings was quantified as the relative difference in area



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

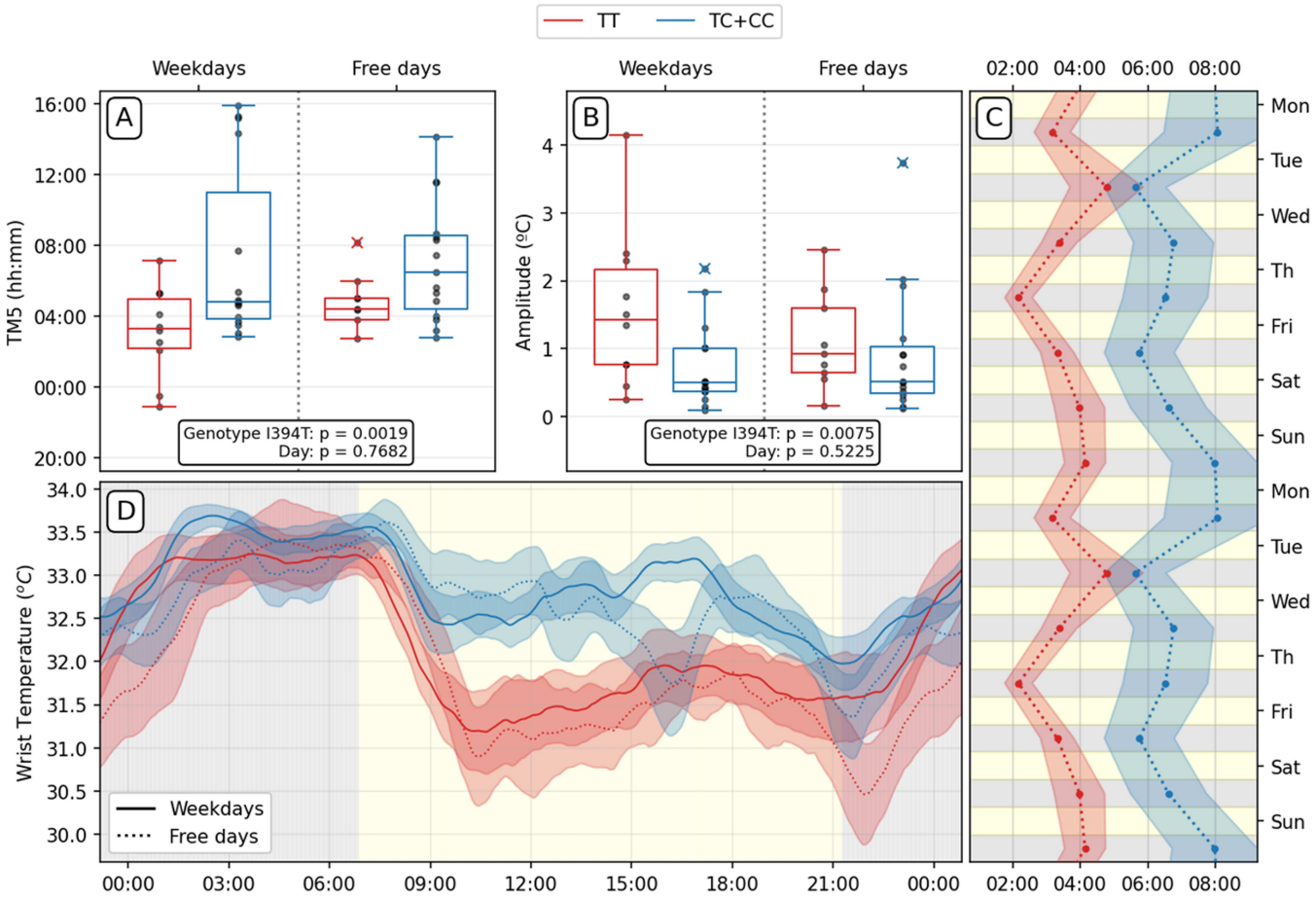


**FIGURE 2** | Analysis of the wrist temperature (WT) ambulatory recordings and phase markers, grouped by the participants' I394T SNP genotype: TT (N = 10, red) and TC + CC (*N* = 15, blue), and by type of day: weekdays (from Sunday 16:00:00 to Friday 15:59:59) and free days (from Friday 16:00:00 to Sunday 15:59:59). (A) Boxplots of per-participant averages of the WT TM5 phase marker. (B) Boxplots of per-participant averages of the WT absolute amplitude. (C) Day-to-day raster plots (mean ± SEM) of the WT TM5 throughout the week (shown twice for ease of reading). (D) Daily mean waves of the WT recordings (mean ± SEM). Individual values and outliers in (A) and (B) are represented as grey points and coloured crosses, respectively. The grey and yellow background shadings in (D) show the actual average natural night- and daytimes during the monitoring, respectively. Wald tests *p*-values of the parameters' statistical significance (for genotype and day) of the LME regression are represented in boxes below the boxplots.

under the curve (AUC) compared to the DIM evening AUC, expressed as a percentage.

Prior to analysis, normality and homoscedasticity were tested using the D'Agostino-Pearson and Bartlett's test, respectively, revealing inconsistent assumptions. Therefore, parametric tests were not used and differences between genotype groups were assessed using Kruskal-Wallis tests.

Finally, a linear mixed-effects model was fitted for each variable (Appendix C) to evaluate the simultaneous influence of genotype and day type on sleep, WT, and m-EDI markers. Since the study was designed to identify novel physiological associations and thus the analyses are exploratory and hypothesis-generating, formal alpha-level corrections (such as Bonferroni) were not applied to the linear mixed-effects models to avoid inflating Type II errors. We advise readers to interpret the resulting p-values as merely descriptive indicators of statistical associations. Analyses were performed in Python v3.10.4 using NumPy v1.22.3, pandas v1.5.1, SciPy v1.8.0, and statsmodels v0.14.0, with their dependencies. Sex was considered as possible confounding factors and thus all analyses were performed accordingly. No significant influence of sex was found on any variable.

## 3 | Results

Overall, I394T SNP exhibited consistent differences across outcomes measured, whereas P10L SNP showed no significant effects. Thus, P10L-related results are presented separately in the Supporting Information S1: Appendix D and Supporting Materials for clarity.

### 3.1 | Genotype Distributions Are in Hardy-Weinberg Equilibrium

Participants (*N* = 25) were genotyped for I394T and P10L SNPs of the *OPN4* gene. For I394T, genotypes were TT = 10, TC = 14, CC = 1 (Minor Allele Frequency, MAF = 32%) and was analysed using a C-allele dominant model (TT vs. TC + CC). For P10L,



1600079x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

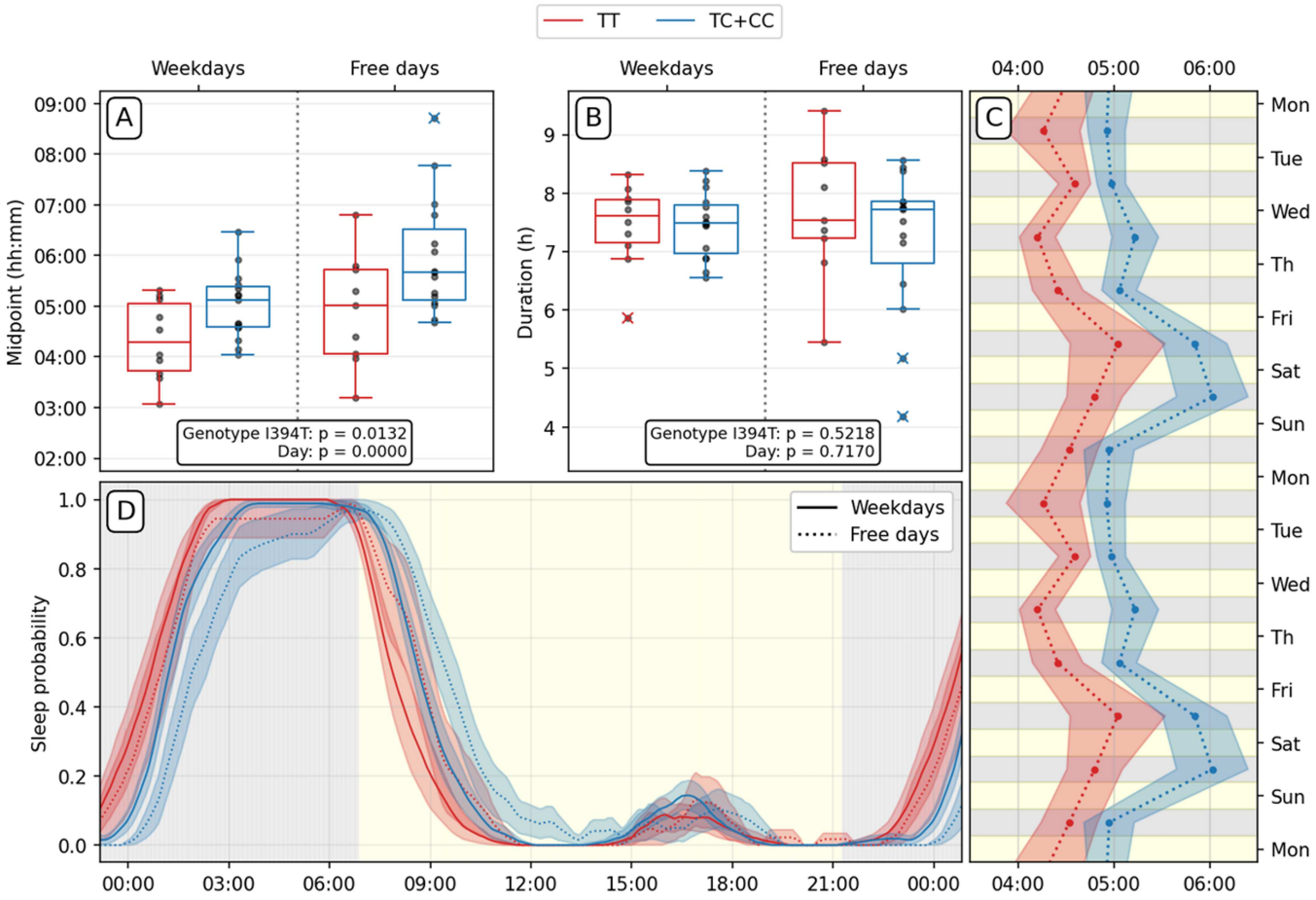


**FIGURE 3** | Analysis of the sleep/wake cycle and its markers, grouped by the participants' I394T SNP genotype: TT (N = 10, red) and TC + CC ($N = 15$, blue), and by type of day: weekdays (from Sunday 16:00:00 to Friday 15:59:59) and free days (from Friday 16:00:00 to Sunday 15:59:59). (A) Boxplots of per-participant averages of the sleep midpoint phase marker. (B) Boxplots of per-participant averages of the sleep duration. (C) Day-to-day raster plots (mean ± SEM) of the sleep midpoints throughout the week (shown twice for ease of reading). (D) Daily mean waves of the sleep probability recordings (mean ± SEM). Individual values and outliers in (A) and (B) are represented as grey points and coloured crosses, respectively. The grey and yellow background shadings in (D) show the actual average natural night- and daytimes during the monitoring, respectively. Wald tests p-values of the parameters' statistical significance (for genotype and day) of the LME regression are represented in boxes below the boxplots.

genotypes were CC = 15, CT = 9, TT = 1 (MAF = 22%), and was analysed with a T-allele dominant model (CC vs. CT + TT). Both SNPs were in Hardy–Weinberg equilibrium ($\chi^2$ test I394T: $p = 0.3577$; P10L: $p = 0.9705$). No significant interaction between SNPs was observed ($\chi^2$ test: $p = 0.3074$; Supporting Table 1).

## 3.2 | Wrist Skin Temperature Daily Rhythm Is Delayed and Attenuated in I394T-C Allele Carriers

A significant phase delay of 3.49 h (SE = 1.12 h; $p = 0.0019$; Table 2) was observed in TC + CC carriers for the WT night phase TM5 (Figure 2A). This delay was accompanied by an attenuation of the absolute WT amplitude in TC + CC than TT (0.65°C ± 0.24 vs. 1.41°C ± 0.21; $p = 0.0075$; Table 2), with 53% versus 10% of participants showing amplitude < 0.5°C (Figure 2B). Tracking the day-to-day progression of the WT phase marker demonstrated that the delay remained stable throughout the entire monitoring week, except for Thursday evening when participants were restricted by the DLMO protocol (Figure 2C). Reduced amplitude in TC + CC daily mean waveforms reflected higher daytime WT rather than lower nocturnal WT (Figure 2D). The type of day did not significantly influence either the phase or the amplitude of the WT ($p > 0.5$; Table 2).

## 3.3 | Habitual Sleep Is Delayed in I394T-C Allele Carriers and on Free Days, Without Decrease in Sleep Duration

Parallel to the WT findings, a significant phase delay ($p = 0.0132$; Table 2; Figure 3A) in the habitual sleep midpoint of the TC + CC group compared with the TT group was observed. This delay did not come at the expense of sleep quantity, since sleep duration was affected neither by genotype nor by type of day, remaining consistently above the recommended threshold of 7 h for healthy adults [44] across all groups (7.42 ± 0.13 h; Table 3; Figure 3B). Similarly to WT, delays were consistent across the week (Figure 3C), and post-prandial naps also occurred at similar times (Figure 3D). Free days showed a 0.82 h delay relative to weekdays (SE = 0.18 h; Table 2), revealing a low-to-moderate baseline level of social jetlag.



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

**TABLE 3** | Average phase and amplitude markers of wrist skin temperature, melanopic equivalent daylight (m-ED) illuminance and sleep, represented as mean (SE). Values are averaged across all participants, TT and TC + CC groups (I394T) and CC and CT + TT (P10L), separately by weekdays (WD) and free days (FD). The overall average of the whole group (top row) and the differences between weekdays and free days (FD-WD) for each group are also shown. Times are represented in h:mm format, whereas durations are represented in decimal hours.

| | | | Wrist Temperature | | Sleep | | m-ED Illuminance | |
|---|---|---|---|---|---|---|---|---|
| | | | TM5 (h:mm) | Amplitude (°C) | Midpoint (h:mm) | Duration (h) | TM10 (h:mm) | Amplitude (melanopic lx) |
| All participants | | Overall | 6:14 (0:49) | 1.04 (0.19) | 4:56 (0:10) | 7.42 (0.13) | 14:17 (0:10) | 209.2 (35.0) |
| | | WD | 5:39 (0:56) | 1.07 (0.19) | 4:44 (0:10) | 7.45 (0.12) | 14:16 (0:10) | 184.7 (28.3) |
| | | FD | 5:47 (0:49) | 1.0 (0.18) | 5:34 (0:15) | 7.38 (0.25) | 15:13 (0:16) | 271.2 (51.8) |
| | | FD-WD | 0:08 (1:06) | −0.06 (0.23) | 0:50 (0:11) | −0.07 (0.23) | 0:58 (0:13) | 86.4 (49.9) |
| I394T | TT | WD | 3:08 (0:49) | 1.57 (0.37) | 4:20 (0:15) | 7.45 (0.22) | 14:00 (0:15) | 211.3 (51.6) |
| | | FD | 3:37 (1:25) | 1.11 (0.24) | 4:55 (0:22) | 7.67 (0.38) | 15:11 (0:31) | 213.1 (59.9) |
| | | FD-WD | 0:29 (1:22) | −0.46 (0.4) | 0:35 (0:15) | 0.22 (0.35) | 1:11 (0:24) | 1.8 (75.2) |
| | TC + CC | WD | 7:20 (1:18) | 0.73 (0.16) | 5:01 (0:10) | 7.45 (0.13) | 14:26 (0:13) | 167.0 (33.1) |
| | | FD | 7:05 (0:52) | 0.94 (0.25) | 5:58 (0:18) | 7.22 (0.32) | 15:14 (0:19) | 306.0 (74.8) |
| | | FD-WD | −0:14 (1:35) | 0.21 (0.26) | 0:57 (0:15) | −0.25 (0.32) | 0:48 (0:16) | 139.0 (64.0) |
| P10L | CC | WD | 5:52 (1:11) | 1.05 (0.29) | 4:46 (0:15) | 7.42 (0.18) | 14:08 (0:15) | 211.9 (44.1) |
| | | FD | 6:30 (0:46) | 0.99 (0.28) | 5:37 (0:19) | 7.62 (0.22) | 15:05 (0:24) | 292.3 (82.5) |
| | | FD-WD | 0:37 (1:17) | −0.06 (0.37) | 0:50 (0:11) | 0.20 (0.23) | 0:58 (0:16) | 80.5 (83.8) |
| | CT + TT | WD | 5:22 (1:34) | 1.09 (0.24) | 4:43 (0:11) | 7.52 (0.17) | 14:25 (0:12) | 150.2 (30.6) |
| | | FD | 4:57 (1:34) | 1.02 (0.22) | 5:32 (0:25) | 7.12 (0.48) | 15:21 (0:22) | 246.2 (60.9) |
| | | FD-WD | −0:26 (1:55) | −0.07 (0.27) | 0:50 (0:21) | −0.40 (0.43) | 0:56 (0:22) | 96.0 (50.0) |

Splitting the cohort by genotype revealed that I394T-C carriers averaged 57 min (SE = 15 min) of social jetlag compared to just 35 min (SE = 15 min) in the TT group (Table 3).

### 3.4 | I394T-C and P10L-T Allele Carriers Do Not Expose to Different Melanopic Illuminance Patterns

The cohort as a whole maintained a well-defined diurnal light schedule (mean TM10 = 14:17 h ± 10 min; Table 3) which closely aligned with the local solar noon (around 14:00 CET) during the monitoring window. TC + CC carriers showed a non-significant tendency for later TM10 (Figure 4A–C), while evening light levels declined sharply around sunset across all groups (Figure 4D). The average m-EDI amplitude for the entire group stood at 209.2 ± 35.0 lx (Table 3), with no genotype differences. A significant difference was identified between free days and weekdays (0.86 ± 0.23 h; Table 2), which mirrored the sleep shift.

### 3.5 | Desynchronization Between Rhythmic Variables Is Greater in I394T-C Allele Carriers and on Free Days

A higher degree of internal desynchronization across wrist skin temperature, sleep probability, and m-EDI in the I394T TC + CC group on both weekdays and free days was consistently observed (Figure 5). Coupling between environmental m-EDI and the two internal variables was the weakest and, reflecting a cumulative 'worst-case scenario', the TC + CC group of the I394T SNP on free days displayed the highest vulnerability, reaching a peak desynchronization index between m-EDI and WT of 0.46, corresponding to a 5.52 h lag.

### 3.6 | I394T-C Allele Correlates With Lower Melatonin Levels in Dim Light Conditions

Under dim light, average melatonin concentrations were significantly lower in the I394T TC + CC group than in the TT group ($p = 0.0059$; Figure 6A, top), with 60% of C-allele carriers (9 out of 15) exhibiting lower average melatonin concentrations than the single lowest participant in the TT group. Weekday melatonin suppression did not differ between genotypes (Figure 6B, left) but free-day suppression was higher in the TC + CC group (Figure 6B, right). In terms of circadian phase markers, the clock time of DLMO itself did not show significant differences between groups (Figure 7, left), but the phase angle of entrainment was significantly delayed ($p = 0.04$) in the TC + CC group compared to the TT group (Figure 7, right). Morning melatonin showed non-significant lower trends in C-allele carriers (Supporting Information S1: Figure S5). No significant differences in $PIPR_6$ (Supporting Information S1: Figure S9, left top row) or basal pupil diameter (left bottom row) were observed between TT and TC + CC carriers.

## 4 | Discussion

The present study provides the first multimodal characterisation of the influence of two human melanopsin gene (*OPN4*)



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

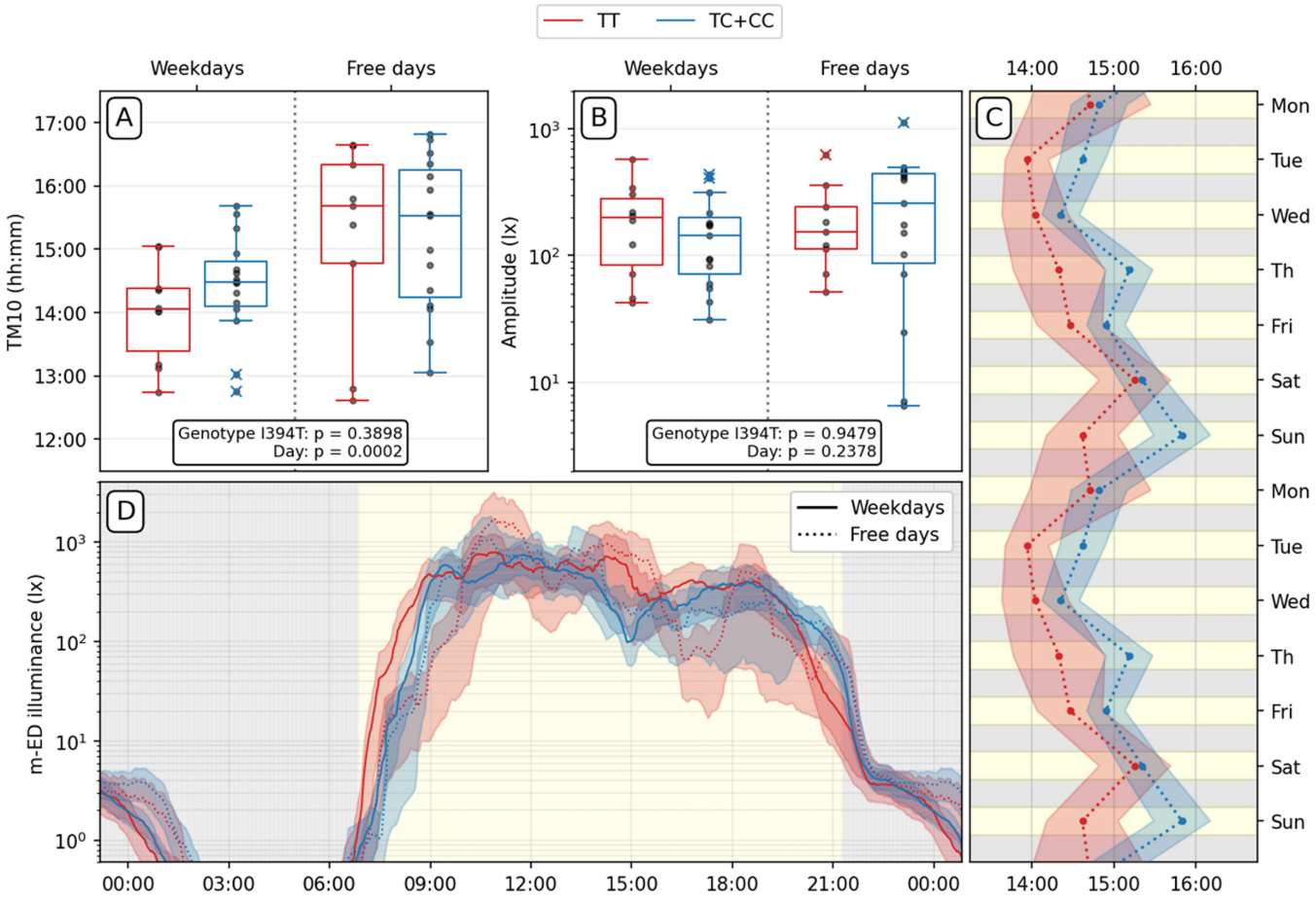


**FIGURE 4** | Analysis of the melanopic equivalent daylight illuminance (m-EDI) ambulatory recordings and markers, grouped by the participants' I394T SNP: TT (N = 10, red) and TC + CC (N = 15, blue), and by type of day: weekdays (from Sunday 16:00:00 to Friday 15:59:59) and free days (from Friday 16:00:00 to Sunday 15:59:59). (A) Boxplots of per-participant averages of the m-EDI TM10 phase marker. (B) Boxplots of per-participant averages of the m-EDI absolute amplitude. (C) Day-to-day raster plots (mean ± SEM) of the m-EDI TM10 phase markers throughout the week (shown twice for ease of reading). (D) Daily mean waves of the m-ED illuminance recordings (mean ± SEM). Individual values and outliers in (A) and (B) are represented as grey points and coloured crosses, respectively. The grey and yellow background shadings in (D) show the actual average natural night- and daytimes during the monitoring, respectively. Wald tests p-values of the parameters' statistical significance (for genotype and day) of the LME regression are represented in boxes below the boxplots.

missense variants -I394T (rs1079610) and P10L (rs2675703)- on non-image-forming physiology in healthy young adults under real-world light exposure. Using ambulatory circadian monitoring, melatonin profiling, and pupillary light responses, we found that the I394T-C allele, but not the P10L-T allele, was associated with delayed sleep, increased phase angle of entrainment, attenuated wrist temperature amplitude, greater internal desynchronization, reduced nocturnal melatonin under dim-light conditions, and enhanced melatonin suppression under free-day conditions, despite similar light exposure profiles. These findings extend previous genetic associations linking I394T to circadian behaviour and physiology [21–23] and indicate distinct functional consequences of melanopsin missense variants.

I394T-C carriers exhibited delayed wrist temperature and sleep rhythms without corresponding shifts in DLMO. This preserved central circadian phase amidst delayed behavioural outputs mirrors non-circadian delayed sleep phase disorder phenotypes reported previously [45] and occurred despite similar melanopic illuminance profiles (Figure 4), suggesting altered sensitivity to light or differences in acute light exposure rather than altered SCN pacemaking. Consistent with this view, mice studies show that melanopsin modulates affective behaviour, mood, and learning via ipRGC projections to mood-regulatory and thalamic circuits [46, 47]. Distinct ipRGC subtypes also project widely beyond the SCN, supporting multiple NIF outputs independent of SCN pacemaking [48, 49]. In another study, ablation of all ipRGCs except the SCN-projecting subset abolished body temperature and sleep responses to acute light, suggesting the SCN is not the sole mediator of acute NIF effects [50]. The wrist temperature rhythm in I394T-C carriers also showed reduced amplitude, reflecting diminished circadian robustness and resembling patterns observed in older cohorts [51]. Greater desynchronization between light exposure, sleep, and wrist temperature, while endogenous rhythms remain relatively coherent, indicates a reduced capacity to phase-lock behavioural rhythms to environmental light, suggesting selective impairment in light-driven entrainment rather than global circadian disruption. These observations align with prior self-report studies linking I394T-C to later chronotype and delayed sleep timing [23].



1600079x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

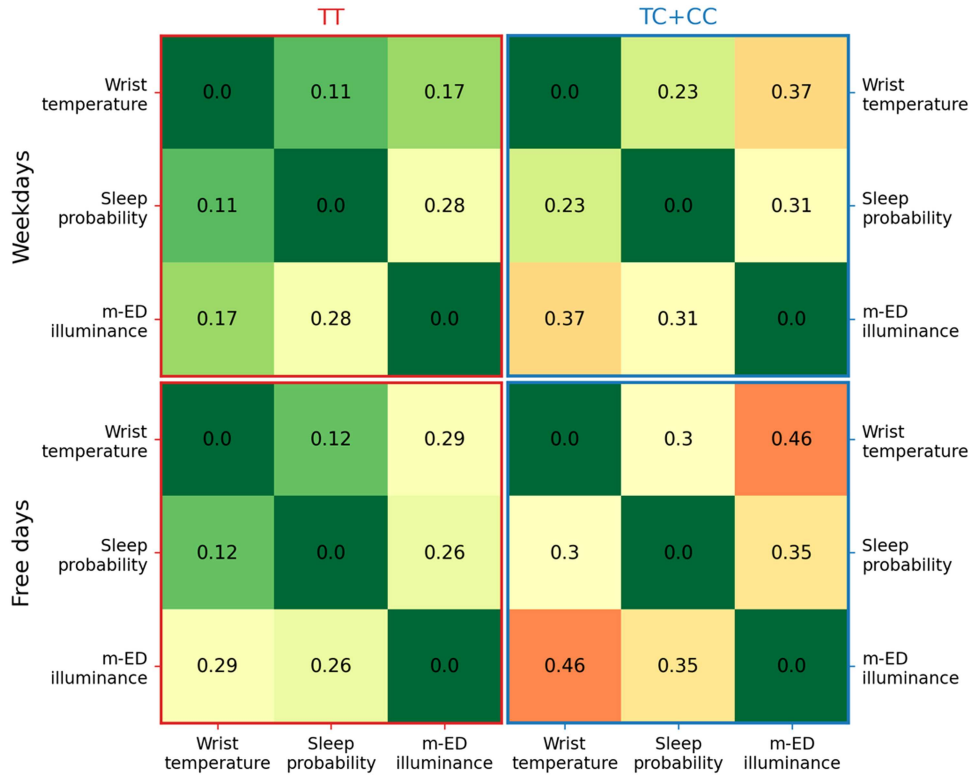


**FIGURE 5** | Desynchronisation matrices between the wrist temperature, sleep probability and melanopic equivalent daylight (m-ED) illuminance ambulatory recordings. Data were grouped by the participants' I394T SNP: TT ($N = 10$, red) and TC + CC ($N = 15$, blue), and by type of day: weekdays (from Sunday 16:00:00 to Friday 15:59:59) and free days (from Friday 16:00:00 to Sunday 15:59:59). Indices shown in the matrices were calculated by normalising in the range [0, 1] the cross-correlation lag between the average daily curves of the variables (see Section 2.4 for a detailed explanation of the calculation method).

I394T-C carriers also displayed lower nocturnal melatonin amplitude under dim-light conditions, consistent with genetically influenced interindividual variability [52], and with ageing-related circadian phenotypes [53–56]. Interestingly, considering the large interindividual variability in melatonin suppression reported in the literature [26, 57, 58], we unexpectedly observed enhanced melatonin suppression on free days in I394T-C carriers despite similar light exposure. Similar findings point out that while melatonin suppression and phase shifts often follow a similar monotonic association, they are functionally independent [59]. These findings suggest that distinct NIF outputs are mediated via separable mechanisms and that I394T can modulate behavioural and hormonal markers independently of core SCN pacemaking. Considering that elevated melatonin enhances thermoregulation (especially distal heat loss) and sleep [60], and reduces sleep onset latency [61], we hypothesize that melatonin variations may contribute to the distinct WT and sleep rhythms observed between I394T-C carriers and non-carriers. Future research should determine whether these differences are driven by the SCN-dependent pathway (modulated by melatonin) or by SCN-independent pathways, which may also critically depend on melatonin.

No genotype-dependent effects were detected on the $PIPR_6$ or on baseline pupil diameter for the I394T SNP. This result stands in contrast to previous observations of dose-dependent relationships between the I394T allele and PLR metrics [21]. This discrepancy likely reflects differences in both stimulus characteristics and metric used, as the present study used a polychromatic 1-s pulse at around 200 lux and the PIPR at 6 s ($PIPR_6$), conditions under which rod and cone inputs substantially contribute to the pupil response and melanopsin-mediated contributions are comparatively weaker [62]. Although PIPR is mediated by ipRGCs [63], the integration of classical photoreceptor inputs within ipRGCs [4–6] implies that genotype-related differences in melanopsin function may be attenuated under the broad-spectrum, moderate-intensity indoor light conditions used in this study, thereby highlighting the necessity of using melanopsin-optimized stimuli for detecting genotype-dependent differences in PLR. However, although polychromatic light is not ideal for dissecting specific mechanistic pathways, it does represent the type of light to which humans are typically exposed in daily life; for this reason, commercially available lamps were used to approximate real-world lighting conditions.

In contrast, the P10L-T allele did not affect any ambulatory, hormonal, or pupillary measures, despite prior links to chronic insomnia [19] and seasonal affective disorder [20]. Previous *in vitro* and *in vivo* studies of both variants have yielded mixed evidence, with functional consequences differing between cellular and organismal contexts [18, 24]. This divergence supports the conclusion that I394T and P10L differ in their physiological impact despite both being missense polymorphisms. Minor allele frequencies (MAF) obtained using the NCBI ALFA project [64] indicate that the I394T-C allele is most prevalent in populations of European origin (35.95%), whereas frequencies are significantly lower in Asian (22.52%–27.88%) and African-



1600079x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

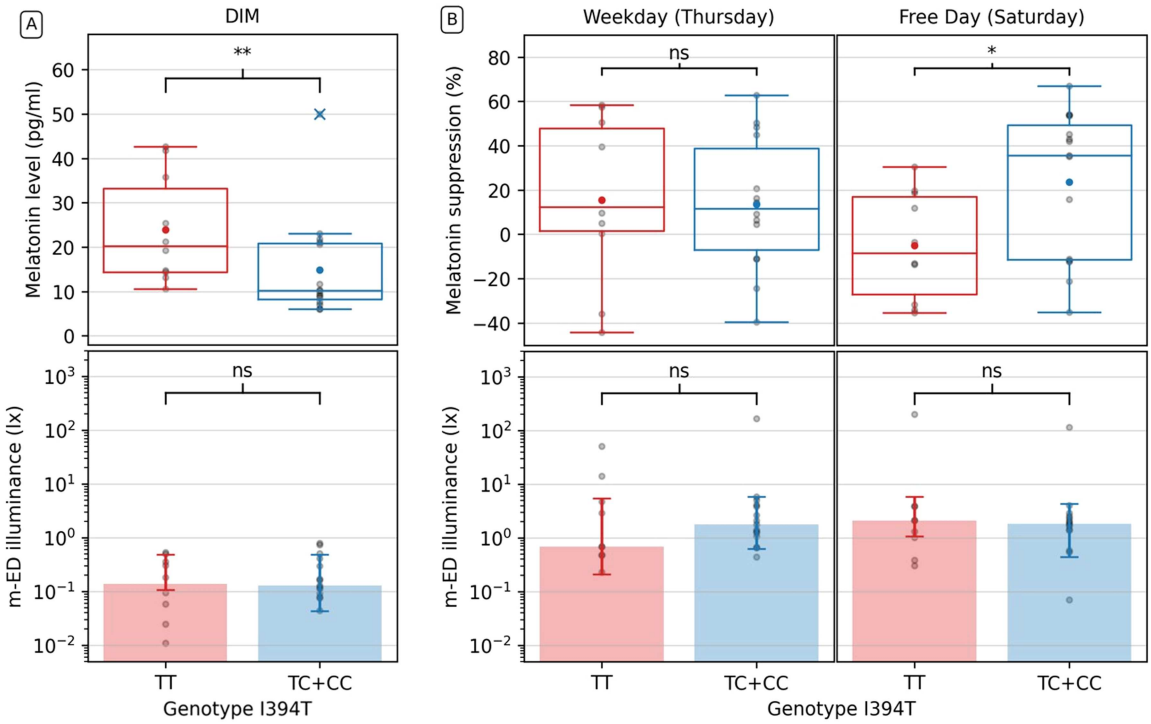


**FIGURE 6** | Boxplots of average melatonin concentration and suppression, and barplots of average melanopic equivalent illuminance (m-EDI), grouped by I394T SNP genotype: TT ($N = 10$, red) and TC + CC ($N = 15$, blue). (A) Average melatonin level (top) and average m-EDI (bottom) on the control evening under dim light conditions. (B) Melatonin suppression (top) and average m-EDI (bottom) on the weekday evening under habitual light conditions (left) and on the free day evening under habitual light conditions (right). Individual averages were calculated from DLMO onwards and are represented as grey dots. Boxplots' means and outliers are represented as coloured circles and crosses, respectively. Melatonin suppression was calculated by AUC relative difference to DIM AUC. To keep consistency with the melatonin boxplots, m-EDI barplots are represented as median ± Q3/Q1 quantiles. Kruskal-Wallis tests were performed between groups for each day and variable (***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: not significant).

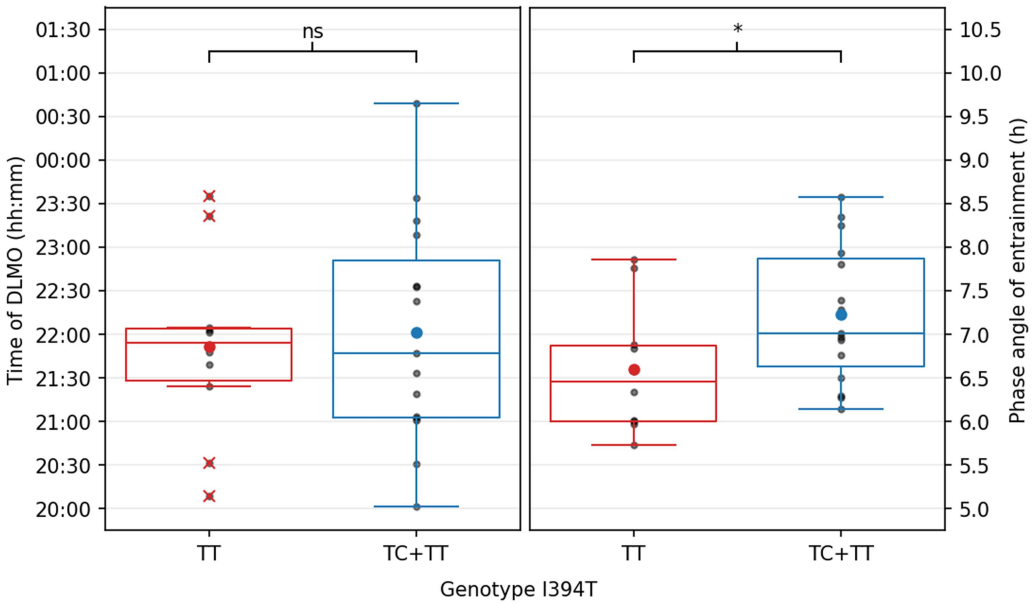


**FIGURE 7** | Dim Light Melatonin Onset (DLMO, left) and phase angle of entrainment, calculated as the time difference between the sleep midpoint and the time of DLMO (right). Participants were grouped by I394T SNP genotype: TT ($N = 10$, red) and TC + CC ($N = 15$, blue). The DLMO was calculated from control evening samples and sleep midpoints were calculated as the average of the whole recording period. Individual values and averages are represented as grey points; means and outliers are represented as coloured circles and crosses, respectively. A Kruskal-Wallis test was performed between the two groups in both cases (***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: not significant).



1600079x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

descendant populations (18.3%–22.79%). This trend is even more pronounced in specific studies of Japanese university students, which report frequencies as low as 11.6%–18.1% [21, 23]. We hypothesise that the differences observed for the I394T variant in our European cohort, where the C-allele frequency reached 34%, may undergo local adaptation to varying latitudes or photoperiods. In contrast, while the P10L-T allele also shows ethnic variability -13.69% in Europeans (NCBI ALFA project) and 22% in our sample, compared to much lower rates in Asians (5.03%–6.91%) and populations form African origin (2.14%–4.36%)-, its associations remain linked to specific clinical groups, such as the 65.5% frequency observed in Mexican insomnia patients [19]. We hypothesise that the phenotypic penetrance of *OPN4* polymorphisms may be influenced by ethnic background and local adaptation, although definitive conclusions regarding environmental or latitudinal selection pressures would require extensive population-genetic mapping, physiological replication across diverse geographical regions, and formal environmental validation.

Even though we did not find any differences between men and women, it is important to note the potential confounding effect of untracked variations in sex steroid hormones. Human core body temperature (CBT) is sensitive to fluctuations in oestrogens and progesterone, with oestrogens generally lowering CBT and progesterone exerting a thermogenic effect. Consequently, there is a sustained increase of 0.3°C to 0.7°C in CBT during the luteal phase compared to the follicular phase, which also reduces the amplitude of the daily CBT rhythm in the former [65]. However, there is conflicting evidence regarding WT, with studies reporting either no differences in WT between the follicular and luteal phases across the circadian cycle [66], or a pattern in which WT peaks during the luteal phase and is lowest during the late follicular phase [67]. Thus, if our TC + CC group had a higher proportion of women in the luteal phase or using oral contraceptives, it remains possible that the reduced WT amplitude observed in this cohort is driven by the menstrual cycle rather than the I394T SNP. Conversely, while lower nocturnal values typically account for differences in CBT amplitude [65], in our cohort the reduced WT amplitude was driven by elevated daytime temperatures (rather than reduced nighttime values), which suggest that the I394T SNP may indeed contribute to the observed reduction in WT amplitude. Similarly, evidence regarding endogenous melatonin levels across the luteal and follicular phases remains conflicting [68], although studies minimising confounding factors through constant routine protocols report no significant differences between phases [69–71]. Finally, given that women exhibit significantly higher melatonin amplitudes than men [72] and that our sample is predominantly female (80%), young, and healthy, it is worth noting that these findings may not may not be generalisable to men, older adults, or peri- and postmenopausal women.

Several limitations warrant consideration. The sample size remains modest for genetic association studies. Furthermore, given the genotype distributions of both SNPs, with only one homozygous carrier for each minor allele, the data had to be analysed using dominant models, which precludes meaningful assessment of gene-dosage or homozygous-specific effects. The PLR protocol was not melanopsin-optimized, and wrist-worn m-EDI measurements may imperfectly capture ocular light exposure [73]. Home-based saliva collection could influence melatonin measurement, and other *OPN4* variants (e.g., R168C, associated with delayed sleep-wake phase disorder [74]) were not assessed. Sex imbalance of the sample (twenty women, five men) may limit generalizability. In addition, we acknowledge that, because menstrual-cycle phases and oral contraceptive use were not controlled for or monitored, the results may be partially confounded by an uneven distribution of participants in the luteal-phase or oral contraceptive use between the genotype groups.

In summary, the I394T-C allele confers a coherent physiological characterised by delayed behavioural rhythms, reduced peripheral amplitude, lower nocturnal melatonin, and increased internal desynchronization, while central circadian phase remains preserved. These results support a broader role for melanopsin in maintaining circadian amplitude, internal coherence, and environmental phase-locking beyond canonical SCN entrainment. In contrast, the P10L-T allele did not produce detectable effects under real-life conditions. Overall, I394T-C may represent a genetic risk factor for reduced circadian resilience, with possible implications for susceptibility to age-related, seasonal, and sleep disorders.

## Author Contributions

Conception or/and design of the work J.A.M., M.A.B.C. and M.A.R. Data collection J.V.M. Hormonal analysis and pupillometry J.V.M. and M.A.B.C. Polymorphism analysis A.P. and J.V.M. Data cleaning, analysis and interpretation J.V.M. Drafted the article JVM. Substantially revised the article J.A.M., M.A.R., M.A.B.C. Funding and resources M.A.R., P.F.A.P. All authors commented on drafts and have approved the final version for submission.


## Acknowledgments

This research was funded by the Ministry of Economy and Competitiveness and the Instituto de Salud Carlos III, through a CIBERFES grant (CB16/10/00239). It was also supported by project PID2022-136577OB-I00, funded by MCIN/AEI/10.13039/501100011033 and FEDER/UE, and by "ERDF A way of making Europe", sponsored by the "European Union" or by the "European Union NextGenerationEU/PRTR". J.V.M. predoctoral contract has been funded by grant '21610/FPI/21' from Fundación Séneca, Región de Murcia (Spain).


## Conflicts of Interest

M.A.R. and J.A.M. are funding partners of Kronohealth SL, a spin-off company participated by the University of Murcia and the company responsible for commercializing the Kronowise device employed in this study. Kronohealth did not financially supported this study nor did pay compensations to the authors. All other authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References


1. C. A. Czeisler, "The Effect of Light on the Human Circadian Pacemaker," in *Circadian Clocks and Their Adjustment*, Ciba Foundation Symposium 183 (John Wiley, 1995), 254–290, https://doi.org/10.1002/9780470514597.ch14.

2. R. Y. Moore and V. B. Eichler, "Loss of a Circadian Adrenal Corticosterone Rhythm Following Suprachiasmatic Lesions in the Rat," *Brain Research* 42 (1972): 201–206, https://doi.org/10.1016/0006-8993(72)90054-6.





16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

3. F. K. Stephan and I. Zucker, "Circadian Rhythms in Drinking Behavior and Locomotor Activity of Rats Are Eliminated by Hypothalamic Lesions," *Proceedings of the National Academy of Sciences* 69 (1972): 1583–1586, https://doi.org/10.1073/pnas.69.6.1583.

4. D. M. Dacey, H.-W. Liao, B. B. Peterson, et al., "Melanopsin-Expressing Ganglion Cells in Primate Retina Signal Colour and Irradiance and Project to the LGN," *Nature* 433 (2005): 749–754, https://doi.org/10.1038/nature03387.

5. K. Y. Wong, F. A. Dunn, D. M. Graham, and D. M. Berson, "Synaptic Influences on Rat Ganglion-Cell Photoreceptors," *Journal of Physiology* 582 (2007): 279–296, https://doi.org/10.1113/jphysiol.2007.133751.

6. D. Göz, K. Studholme, D. A. Lappi, M. D. Rollag, I. Provencio, and L. P. Morin, "Targeted Destruction of Photosensitive Retinal Ganglion Cells With a Saporin Conjugate Alters the Effects of Light on Mouse Circadian Rhythms," *PLoS One* 3 (2008): e3153, https://doi.org/10.1371/journal.pone.0003153.

7. D. M. Berson, F. A. Dunn, and M. Takao, "Phototransduction by Retinal Ganglion Cells That Set the Circadian Clock," *Science* 295 (2002): 1070–1073, https://doi.org/10.1126/science.1067262.

8. S. Hattar, H.-W. Liao, M. Takao, D. M. Berson, and K.-W. Yau, "Melanopsin-Containing Retinal Ganglion Cells: Architecture, Projections, and Intrinsic Photosensitivity," *Science* 295 (2002): 1065–1070, https://doi.org/10.1126/science.1069609.

9. R. J. Lucas, S. Hattar, M. Takao, D. M. Berson, R. G. Foster, and K.-W. Yau, "Diminished Pupillary Light Reflex at High Irradiances in Melanopsin-Knockout Mice," *Science* 299 (2003): 245–247, https://doi.org/10.1126/science.1077293.

10. P. D. R. Gamlin, D. H. McDougal, J. Pokorny, V. C. Smith, K.-W. Yau, and D. M. Dacey, "Human and Macaque Pupil Responses Driven by Melanopsin-Containing Retinal Ganglion Cells," *Vision Research* 47 (2007): 946–954, https://doi.org/10.1016/j.visres.2006.12.015.

11. K. Thapan, J. Arendt, and D. J. Skene, "An Action Spectrum for Melatonin Suppression: Evidence for a Novel Non-Rod, Non-Cone Photoreceptor System in Humans," *Journal of Physiology* 535 (2001): 261–267, https://doi.org/10.1111/j.1469-7793.2001.t01-1-00261.x.

12. G. C. Brainard, J. P. Hanifin, J. M. Greeson, et al., "Action Spectrum for Melatonin Regulation in Humans: Evidence for a Novel Circadian Photoreceptor," *Journal of neuroscience* 21 (2001): 6405–6412, https://doi.org/10.1523/JNEUROSCI.21-16-06405.2001.

13. J. J. Gooley, S. M. W. Rajaratnam, G. C. Brainard, R. E. Kronauer, C. A. Czeisler, and S. W. Lockley, "Spectral Responses of the Human Circadian System Depend on the Irradiance and Duration of Exposure to Light," *Science Translational Medicine* 2 (2010): 31ra33, https://doi.org/10.1126/scitranslmed.3000741.

14. C. Cajochen, M. Münch, S. Kobialka, et al., "High Sensitivity of Human Melatonin, Alertness, Thermoregulation, and Heart Rate to Short Wavelength Light," *Journal of Clinical Endocrinology and Metabolism* 90 (2005): 1311–1316, https://doi.org/10.1210/jc.2004-0957.

15. K. Kräuchi, C. Cajochen, E. Werth, and A. Wirz-Justice, "Warm Feet Promote the Rapid Onset of Sleep," *Nature* 401 (1999): 36–37, https://doi.org/10.1038/43366.

16. E. Ortiz-Tudela, A. Martinez-Nicolas, J. Albares, et al., "Ambulatory Circadian Monitoring (Acm) Based on Thermometry, Motor Activity and Body Position (TAP): A Comparison With Polysomnography," *Physiology & Behavior* 126 (2014): 30–38, https://doi.org/10.1016/j.physbeh.2013.12.009.

17. K. A. Roecklein, P. M. Wong, P. L. Franzen, et al., "Melanopsin Gene Variations Interact With Season to Predict Sleep Onset and Chronotype," *Chronobiology International* 29 (2012): 1036–1047, https://doi.org/10.3109/07420528.2012.706766.

18. J. Rodgers, S. Hughes, C. A. Pothecary, et al., "Defining the Impact of Melanopsin Missense Polymorphisms Using In Vivo Functional Rescue," *Human Molecular Genetics* 27 (2018): 2589–2603, https://doi.org/10.1093/hmg/ddy150.

19. B. E. Gutiérrez-Amavizca, E. Prado Montes De Oca, J. P. Gutiérrez-Amavizca, et al., "Association of P10L Polymorphism in Melanopsin Gene With Chronic Insomnia in Mexicans," *International Journal of Environmental Research and Public Health* 18 (2021): 571, https://doi.org/10.3390/ijerph18020571.

20. K. A. Roecklein, K. J. Rohan, W. C. Duncan, et al., "A Missense Variant (P10L) of the Melanopsin (OPN4) Gene in Seasonal Affective Disorder," *Journal of Affective Disorders* 114 (2009): 279–285, https://doi.org/10.1016/j.jad.2008.08.005.

21. S. Higuchi, A. Hida, S. Tsujimura, et al., "Melanopsin Gene Polymorphism I394T Is Associated With Pupillary Light Responses in a Dose-Dependent Manner," *PLoS One* 8 (2013): e60310, https://doi.org/10.1371/journal.pone.0060310.

22. K. Roecklein, P. Wong, N. Ernecoff, et al., "The Post Illumination Pupil Response Is Reduced in Seasonal Affective Disorder," *Psychiatry Research* 210 (2013): 150–158, https://doi.org/10.1016/j.psychres.2013.05.023.

23. S. Lee, A. Hida, S. Kitamura, K. Mishima, and S. Higuchi, "Association Between the Melanopsin Gene Polymorphism OPN4*Ile394Thr and Sleep/Wake Timing in Japanese University Students," *Journal of physiological anthropology* 33 (2014): 9, https://doi.org/10.1186/1880-6805-33-9.

24. J. Rodgers, S. N. Peirson, S. Hughes, and M. W. Hankins, "Functional Characterisation of Naturally Occurring Mutations in Human Melanopsin," *Cellular and Molecular Life Sciences* 75 (2018): 3609–3624, https://doi.org/10.1007/s00018-018-2813-0.

25. F. G. Amaral and J. Cipolla-Neto, "A Brief Review About Melatonin, a Pineal Hormone," *Archives of Endocrinology and Metabolism* 62 (2018): 472–479, https://doi.org/10.20945/2359-3997000000066.

26. A. J. K. Phillips, P. Vidafar, A. C. Burns, et al., "High Sensitivity and Interindividual Variability in the Response of the Human Circadian System to Evening Light," *Proceedings of the National Academy of Sciences* 116 (2019): 12019–12024, https://doi.org/10.1073/pnas.1901824116.

27. R. J. Lucas, S. N. Peirson, D. M. Berson, et al., "Measuring and Using Light in the Melanopsin Age," *Trends in Neurosciences* 37 (2014): 1–9, https://doi.org/10.1016/j.tins.2013.10.004.

28. M. A. Bonmati-Carrion, B. Middleton, V. Revell, D. J. Skene, M. A. Rol, and J. A. Madrid, "Circadian Phase Asessment by Ambulatory Monitoring in Humans: Correlation With Dim Light Melatonin Onset," *Chronobiology International* 31 (2014): 37–51, https://doi.org/10.3109/07420528.2013.820740.

29. S. Ancoli-Israel, R. Cole, C. Alessi, M. Chambers, W. Moorcroft, and C. P. Pollak, "The Role of Actigraphy in the Study of Sleep and Circadian Rhythms," *Sleep* 26 (2003): 342–392, https://doi.org/10.1093/sleep/26.3.342.

30. T. Roenneberg, T. Kantermann, M. Juda, C. Vetter, and K. V. Allebrandt, *Handbook of Experimental Pharmacology* (Springer, 2013), 311–331.

31. E. R. Stothard, A. W. McHill, C. M. Depner, et al., "Circadian Entrainment to the Natural Light-Dark Cycle Across Seasons and the Weekend," *Current Biology* 27 (2017): 508–513, https://doi.org/10.1016/j.cub.2016.12.041.

32. A. Martinez-Nicolas, M. Meyer, S. Hunkler, et al., "Daytime Variation in Ambient Temperature Affects Skin Temperatures and Blood Pressure: Ambulatory Winter/Summer Comparison in Healthy Young Women," *Physiology & Behavior* 149 (2015): 203–211, https://doi.org/10.1016/j.physbeh.2015.06.014.

33. K. Dugan, H. Lawrence, D. Hares, C. Fisher, and B. Budowle, "An Improved Method for Post-Pcr Purification for mtDNA Sequence Analysis," *Journal of Forensic Sciences* 47 (2002): 1–8.



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

34. C. J. Madrid-Navarro, F. Escamilla-Sevilla, A. Mínguez-Castellanos, et al., "Multidimensional Circadian Monitoring by Wearable Biosensors in Parkinson's Disease," *Frontiers in Neurology* 9 (2018): 157, https://doi.org/10.3389/fneur.2018.00157.

35. C. J. Madrid-Navarro, F. J. Puertas Cuesta, F. Escamilla-Sevilla, et al., "Validation of a Device for the Ambulatory Monitoring of Sleep Patterns: A Pilot Study on Parkinson's Disease," *Frontiers in Neurology* 10 (2019): 356, https://doi.org/10.3389/fneur.2019.00356.

36. J. Vicente-Martínez, P. F. Almaida-Pagan, A. Martinez-Nicolas, J. A. Madrid, M.-A. Rol, and M.-Á. Bonmatí-Carrión, *Statistical Methods at the Forefront of Biomedical Advances*. Springer International Publishing, 2023), 185–236.

37. E. Ortiz-Tudela, P. F. Innominato, M. A. Rol, F. Lévi, and J. A. Madrid, "Relevance of Internal Time and Circadian Robustness for Cancer Patients," *BMC Cancer* 16 (2016): 285, https://doi.org/10.1186/s12885-016-2319-9.

38. M. A. Bonmati-Carrion, K. Hild, C. Isherwood, et al., "Relationship Between Human Pupillary Light Reflex and Circadian System Status," *PLoS One* 11 (2016): e0162476, https://doi.org/10.1371/journal.pone.0162476.

39. S. W. Lockley, "Journal of Pineal Research Guideline for Authors: Measuring Melatonin in Humans," *Journal of Pineal Research* 69 (2020): e12664, https://doi.org/10.1111/jpi.12664.

40. A. Voultsios, D. J. Kennaway, and D. Dawson, "Salivary Melatonin as a Circadian Phase Marker: Validation and Comparison to Plasma Melatonin," *Journal of Biological Rhythms* 12 (1997): 457–466, https://doi.org/10.1177/074873049701200507.

41. A. J. Zele, B. Feigl, S. S. Smith, and E. L. Markwell, "The Circadian Response of Intrinsically Photosensitive Retinal Ganglion Cells," *PLoS One* 6 (2011): e17860, https://doi.org/10.1371/journal.pone.0017860.

42. M. Münch, L. Léon, S. V. Crippa, and A. Kawasaki, "Circadian and Wake-Dependent Effects on the Pupil Light Reflex in Response to Narrow-Bandwidth Light Pulses," *Investigative Opthalmology & Visual Science* 53 (2012): 4546–4555, https://doi.org/10.1167/iovs.12-9494.

43. C. Kelbsch, T. Strasser, Y. Chen, et al., "Standards in Pupillography," *Frontiers in Neurology* 10 (2019): 129, https://doi.org/10.3389/fneur.2019.00129.

44. N. F. Watson, M. S. Badr, G. Belenky, et al., "Recommended Amount of Sleep for a Healthy Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society," *Sleep* 38 (2015): 843–844, https://doi.org/10.5665/sleep.4716.

45. J. M. Murray, T. L. Sletten, M. Magee, et al., "Prevalence of Circadian Misalignment and Its Association With Depressive Symptoms in Delayed Sleep Phase Disorder," *Sleep* 40 (2017): zsw002, https://doi.org/10.1093/sleep/zsw002.

46. D. C. Fernandez, P. M. Fogerson, L. Lazzerini Ospri, et al., "Light Affects Mood and Learning Through Distinct Retina-Brain Pathways," *Cell* 175 (2018): 71–84.e18, https://doi.org/10.1016/j.cell.2018.08.004.

47. T. A. LeGates, C. M. Altimus, H. Wang, et al., "Aberrant Light Directly Impairs Mood and Learning Through Melanopsin-Expressing Neurons," *Nature* 491 (2012): 594–598, https://doi.org/10.1038/nature11673.

48. C. Beier, Z. Zhang, M. Yurgel, and S. Hattar, "Projections of Iprgcs and Conventional Rgcs to Retinorecipient Brain Nuclei," *Journal of Comparative Neurology* 529 (2021): 1863–1875, https://doi.org/10.1002/cne.25061.

49. J. L. Ecker, O. N. Dumitrescu, K. Y. Wong, et al., "Melanopsin-Expressing Retinal Ganglion-Cell Photoreceptors: Cellular Diversity and Role in Pattern Vision," *Neuron* 67 (2010): 49–60, https://doi.org/10.1016/j.neuron.2010.05.023.

50. A. C. Rupp, M. Ren, C. M. Altimus, et al., "Distinct Iprgc Subpopulations Mediate Light's Acute and Circadian Effects on Body Temperature and Sleep," *eLife* 8 (2019): e44358, https://doi.org/10.7554/eLife.44358.

51. S. A. Rahman, R. M. Gathungu, V. R. Marur, et al., "Age-Related Changes in Circadian Regulation of the Human Plasma Lipidome," *Communications Biology* 6 (2023): 756, https://doi.org/10.1038/s42003-023-05102-8.

52. H. J. Burgess and L. F. Fogg, "Individual Differences in the Amount and Timing of Salivary Melatonin Secretion," *PLoS One* 3 (2008): e3055, https://doi.org/10.1371/journal.pone.0003055.

53. J. N. Zhou, R. Y. Liu, J. Van Heerikhuize, M. A. Hofman, and D. F. Swaab, "Alterations in the Circadian Rhythm of Salivary Melatonin Begin During Middle-Age," *Journal of Pineal Research* 34 (2003): 11–16, https://doi.org/10.1034/j.1600-079X.2003.01897.x.

54. C. García Cobarro, L. Ignez Soares, Y. Kutsenko, and A. Tomas-Loba, "Circadian System and Aging: Where Both Times Interact," *Frontiers in Aging* 6 (2025): 1646794, https://doi.org/10.3389/fragi.2025.1646794.

55. R. Hardeland, "Melatonin and Circadian Oscillators in Aging - A Dynamic Approach to the Multiply Connected Players." *Interdisciplinary Topics in Gerontology and Geriatrics*. A. I. Yashin, S. M. Jazwinski, I. S. B. N., S. Karger AG. 40 (2015), 128–140

56. R. Hardeland, J. A. Madrid, D. X. Tan, and R. J. Reiter, "Melatonin, the Circadian Multioscillator System and Health: The Need for Detailed Analyses of Peripheral Melatonin Signaling," *Journal of Pineal Research* 52 (2012): 139–166, https://doi.org/10.1111/j.1600-079X.2011.00934.x.

57. S. Higuchi, Y. Motohashi, T. Maeda, and K. Ishibashi, "Relationship Between Individual Difference in Melatonin Suppression by Light and Habitual Bedtime," *Journal of Physiological Anthropology and Applied Human Science* 24 (2005): 419–423, https://doi.org/10.2114/jpa.24.419.

58. F. Fazlali, R. Lazar, F. Yahya, O. Stefani, M. Spitschan, and C. Cajochen, "Sex and Seasonal Variations in Melatonin Suppression and Alerting Response to Light," *Journal of the Endocrine Society* 9 (2025): bvaf155, https://doi.org/10.1210/jendso/bvaf155.

59. S. A. Rahman, M. A. St Hilaire, C. Gronfier, et al., "Functional Decoupling of Melatonin Suppression and Circadian Phase Resetting in Humans," *Journal of Physiology* 596 (2018): 2147–2157, https://doi.org/10.1113/JP275501.

60. K. Kräuchi, C. Cajochen, M. Pache, J. Flammer, and A. Wirz-Justice, "Thermoregulatory Effects of Melatonin in Relation to Sleepiness," *Chronobiology International* 23 (2006): 475–484, https://doi.org/10.1080/07420520500545854.

61. E. Ferracioli-Oda, A. Qawasmi, and M. H. Bloch, "Meta-Analysis: Melatonin for the Treatment of Primary Sleep Disorders," *PLoS One* 8 (2013): e63773, https://doi.org/10.1371/journal.pone.0063773.

62. J. J. Gooley, I. Ho Mien, M. A. St. Hilaire, et al., "Melanopsin and Rod–Cone Photoreceptors Play Different Roles in Mediating Pupillary Light Responses During Exposure to Continuous Light in Humans," *Journal of neuroscience* 32 (2012): 14242–14253, https://doi.org/10.1523/JNEUROSCI.1321-12.2012.

63. P. Adhikari, B. Feigl, and A. J. Zele, "Rhodopsin and Melanopsin Contributions to the Early Redilation Phase of the Post-Illumination Pupil Response (PIPR)," *PLoS One* 11 (2016): e0161175, https://doi.org/10.1371/journal.pone.0161175.

64. L. Phan, Y. Jin, H. Zhang, et al., "ALFA," *Allele Frequency Aggregator Available online* (accessed on 14 March 2026), www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/.

65. F. C. Baker, F. Siboza, and A. Fuller, "Temperature Regulation in Women: Effects of the Menstrual Cycle," *Temperature: Multidisciplinary Biomedical Journal* 7 (2020): 226–262, https://doi.org/10.1080/23328940.2020.1735927.

66. A. Shechter, P. Boudreau, F. Varin, and D. B. Boivin, "Predominance of Distal Skin Temperature Changes at Sleep Onset Across



16000793x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Menstrual and Circadian Phases," *Journal of Biological Rhythms* 26 (2011): 260–270, https://doi.org/10.1177/0748730411404677.

67. G. Lin, J. Y. Li, K. Christofferson, S. N. Patel, K. N. Truong, and A. Mariakakis, "Understanding Wrist Skin Temperature Changes to Hormone Variations Across the Menstrual Cycle," *npj Women's Health* 2 (2024): 35, https://doi.org/10.1038/s44294-024-00037-9.

68. F. C. Baker and H. S. Driver, "Circadian Rhythms, Sleep, and the Menstrual Cycle," *Sleep Medicine* 8 (2007): 613–622, https://doi.org/10.1016/j.sleep.2006.09.011.

69. K. P. Wright and P. Badia, "Effects of Menstrual Cycle Phase and Oral Contraceptives on Alertness, Cognitive Performance, and Circadian Rhythms During Sleep Deprivation," *Behavioural Brain Research* 103 (1999): 185–194, https://doi.org/10.1016/s0166-4328(99)00042-x.

70. K. Shibui, M. Uchiyama, M. Okawa, et al., "Diurnal Fluctuation of Sleep Propensity and Hormonal Secretion Across the Menstrual Cycle," *Biological Psychiatry* 48 (2000): 1062–1068, https://doi.org/10.1016/S0006-3223(00)00912-4.

71. S. A. Rahman, L. K. Grant, J. J. Gooley, S. M. W. Rajaratnam, C. A. Czeisler, and S. W. Lockley, "Endogenous Circadian Regulation of Female Reproductive Hormones," *Journal of Clinical Endocrinology & Metabolism* 104 (2019): 6049–6059, https://doi.org/10.1210/jc.2019-00803.

72. S. W. Cain, C. F. Dennison, J. M. Zeitzer, et al., "Sex Differences in Phase Angle of Entrainment and Melatonin Amplitude in Humans," *Journal of Biological Rhythms* 25 (2010): 288–296, https://doi.org/10.1177/0748730410374943.

73. R. Arguelles-Prieto, M.-A. Bonmati-Carrion, M. A. Rol, and J. A. Madrid, "Determining Light Intensity, Timing and Type of Visible and Circadian Light From an Ambulatory Circadian Monitoring Device," *Frontiers in Physiology* 10 (2019): 822, https://doi.org/10.3389/fphys.2019.00822.

74. S. P. Smieszek, C. M. Polymeropoulos, G. Birznieks, and M. H. Polymeropoulos, "Case Report: A Novel Missense Variant in Melanopsin Associates With Delayed Sleep Phenotype: Whole Genome Sequencing Study," *Frontiers in Genetics* 13 (2022): 896192, https://doi.org/10.3389/fgene.2022.896192.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section.
**Supporting File:** jpi70181-sup-0001-supplementary_material.pdf.



1600079x, 2026, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jpi.70181 by Servicio Murciano De Salud, Wiley Online Library on [27/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License