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Inès Daguët, Véronique Raverot, Didier Bouhassira, Claude Gronfier. Circadian rhythmicity of pain sensitivity in humans. *Brain - A Journal of Neurology*, 2022, 145 (9), pp.3225-3235. 10.1093/brain/awac147. hal-03651567v2

HAL Id: hal-03651567

<https://hal.science/hal-03651567v2>

Submitted on 9 Sep 2022

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Circadian rhythmicity of pain sensitivity in humans

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This document/manuscript is the *un-refereed Author's Original Version* (preprint) submitted to the journal *Brain*.

The printed/final version of the article can be found on the journal's website:

<https://academic.oup.com/brain/advance-article-abstract/doi/10.1093/brain/awac147/6637506>

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Running title: Circadian rhythmicity of human pain

Keywords: Pain; circadian; rhythm; sleep; homeostasis

Abbreviations:

CT = Circadian Time

CR = Constant Routine

IRC = Intensity Response Curve

ET₅₀ = Half maximal effective temperature

SCN = Suprachiasmatic nuclei

VAS = Visual Analog Scale

Abstract

Pain intensity has been reported to fluctuate during the day in some experimental and clinical conditions, but the mechanisms underlying these fluctuations are unknown. Although the circadian timing system is known to regulate a wide range of physiological functions, its implication in pain regulation is unknown.

Using highly controlled laboratory constant routine conditions, we show that pain sensitivity is rhythmic over the 24-hours and strongly controlled by the endogenous circadian timing system. We found that the circadian component of pain sensitivity can be modelled with a sinusoidal function, with a maximum in the middle of the night and a minimum in the afternoon. We also found a weak homeostatic control of pain sensitivity, with a linear increase over the 34 hours of prolonged wakefulness, which slowly builds up with sleep pressure. Using mathematical modelling, we describe that the circadian system accounts for ~80% of the full magnitude of pain sensitivity over the 24 hours, and that sleep-related processes account for only ~20%.

Overall, our data reveal that the neurobiological mechanisms involved in driving the rhythmicity of pain perception in humans, with the oscillating time-piece located in the suprachiasmatic nuclei. Our findings highlight the need to consider the time of day in pain assessment, and suggest that personalized circadian medicine may be a promising approach to pain management.

Introduction

Pain intensity has been reported to fluctuate during the day in a number of clinical conditions ¹. The cyclic nature of some headaches ^{2,3} and the diurnal variation of pain related to osteoarthritis are classical clinical observations ^{4,5}. The mechanisms underlying these fluctuations, however, are unknown. In particular, it remains unclear whether such daily variations are related to the internal circadian clock, or to behavioral or environmental factors, such as the sleep/wake cycle or the rest-activity cycle.

Pain has two main interconnected components: a sensory-discriminative component (location, quality, duration, intensity etc.) and an emotional component (unpleasantness, anxiety, motivation, etc.) ⁶. This multidimensional nociceptive response involves the activation of numerous subcortical and cortical regions of the brain (e.g. somatosensory cortices, insula, thalamus, prefrontal cortex), often referred to as the “pain matrix” ⁷. These structures are known to be regulated by the sleep/wake cycle or the circadian clock ⁸⁻¹⁰, but it remains unclear whether pain sensitivity is rhythmic and how it is regulated. The circadian timekeeping system plays a key role in physiology by regulating the rhythmicity of numerous functions, from gene expression to cortical activity and behavioral functions ^{8,9,11-14}. It is, therefore, also likely to be involved in pain perception. The surprising lack of knowledge about the rhythmicity of pain sensitivity may result from the impact of timing on pain perception rarely having been taken into account ¹, and the use of inappropriate protocols for the exploration of pain rhythmicity from a neurobiological and mechanistic point of view. The experimental studies performed to date to investigate pain sensitivity changes during the day in healthy individuals have reported conflicting results ¹⁵⁻²⁰. Indeed, half these articles reported no statistically significant diurnal change in pain perception¹⁸⁻²⁰ and the other half ¹⁵⁻¹⁷ reported a time-of-day effect on pain sensitivity but with a large variation in the timing of maximal pain (between 18:00 and 2:00). In both experimental and clinical studies, the limited number of measurements and their timing (mostly during the daytime) made it impossible to demonstrate unequivocally the existence of a 24-hour rhythmicity in pain sensation. It

is also impossible to determine the origin of any rhythmicity in pain from these studies, because neither of the two types of highly controlled laboratory protocols (constant-routine and forced-desynchrony paradigms) capable of separating endogenous and exogenous rhythms were used^{21,22}. Endogenous rhythms are controlled by the central biological clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus, and exogenous rhythms depend on behavioral or environmental changes, such as the sleep/wake cycle, the dark/light cycle, or the rest/activity cycle. In real-life conditions, endogenous and exogenous influences are expressed simultaneously, making it impossible to attribute rhythmicity to one or the other. In this study, we aimed to determine whether sensitivity to heat pain displays rhythmicity over the 24-h day, and to assess the precise contribution of the circadian clock and sleep-related processes, by systematically assessing pain sensation and gold-standard markers of circadian rhythmicity in highly controlled constant-routine conditions.

Methods

Participants

Twelve healthy men (20 - 29 years old, mean age = 22.7 ± 3.3 years; BMI = 21.8 ± 3.1 kg/m²; height = 1.78 ± 0.07 m; weight = 69.3 ± 12.7 kg) were included in this study. Neurological, psychiatric and sleep disorders were excluded by clinical examination and psychological questionnaires (Pittsburg Sleep Quality Index Questionnaire and Beck Depression Inventory)^{23,24}. Participants had an intermediate chronotype (Mean Horne and Ostberg score = 53.5 ± 5.7)²⁵ and had not done any shift work, or experienced transmeridian travel during the previous three months. Participants had normal visual acuity (Landolt Ring Test and Monoyer scale), contrast vision (Functional Acuity Contrast Test) and color vision (Farnworth D-15 and Ishihara Color Test). All experimental procedures were carried out in accordance with the Declaration of Helsinki. The study was approved by the local research

ethics committee (CPP Lyon Sud-Est II) and participants provided written informed consent for participation.

Study design

Participants were asked to maintain a regular sleep/wake schedule (bedtimes and waketimes within \pm 30 minutes of self-targeted times) for an average of three weeks before admission to the laboratory, with verification by wrist activity and light exposure recordings (ActTrust, Condor Instruments, São Paulo, Brazil). Subjects were then admitted to the laboratory for a 56-hour experimental protocol (Figure 1), in which they were kept in an environment free from external time cues (clocks, television, smartphones, internet, visitors, sunlight etc.). Subjects maintained contact with staff members specifically trained to avoid communicating time-of-day information or the nature of the experimental conditions to the subjects. Participants arrived at about 10:00 on the first day. They were allowed to familiarize themselves with the laboratory environment, low light levels (< 0.5 lux), equipment, and measurements. Lunch and dinner were served at about 12:30 and 19:00. A series of measurements were then performed until bedtime (participant's habitual bedtime), and an 8-hour sleep episode was scheduled (constant darkness; recumbent position). This was followed by a 34-hour constant-routine protocol beginning at the participant's usual waketime on day 2, and ending on day 3 (18:00 on average). Habitual bedtimes were determined on the basis of sleep times averaged over the seven days preceding the laboratory segment of the protocol. Average bedtime was 23:45 and average waketime was 8:00.

Constant routine protocol

A constant routine (CR) paradigm was used to reveal the endogenous circadian rhythmicity of various parameters. The CR was conducted under constant environmental conditions, to eliminate, or distribute across the circadian cycle, the physiological responses evoked by environmental or behavioral stimuli

(i.e. sleeping, eating, changes in posture, light intensity variations)^{21,26}. In practical terms, participants were asked to remain awake for 34 hours (starting at their habitual waketime), with minimal physical activity, while lying in a semi-recumbent (45 °) posture in bed. This posture was also maintained for the collection of urine samples and bowel movements. Room temperature (mean = 23 °C ± 0.6 (SD)) and ambient very dim halogen light levels were kept constant. Light intensity was homogeneous in the room (< 0.5 lux at the participant's eye level in all directions of gaze). Participants were given small equicaloric snacks and fluids at hourly intervals, to maintain an equal nutritional caloric intake and stable hydration over the circadian cycle. Caloric requirements were calculated on the basis of basal metabolic rate determined with the Wilmore nomogram and were adjusted upward by a 7 % activity factor^{27,28}. Fluid intake was calculated for each subject, to account for the sedentary nature of the CR²⁸, and consisted only of water (no caffeinated beverages). A member of the study staff remained in the room with the participant at all times during the CR, to monitor wakefulness and to ensure compliance with the study procedures.

Heat and pain evaluation

Thermal stimuli were applied to the forearm with a Peltier-type thermode (30 × 35 mm) connected to a thermotest device (Somedic AB, Stockholm, Sweden). Heat detection and pain thresholds were determined according to the method of limits (mean of three measurements).

Thermode temperature was gradually increased from a baseline temperature of 32 °C, at a rate of 1 °C/s, and participants were asked to stop the increase in temperature when they started to feel a warm sensation (detection threshold) or a pain sensation (pain threshold). At this point, the temperature returned to baseline at a rate of 1 °C/s. A minimum interval of 20 s was respected between each threshold measurement. If participants had not pressed the button by the time the maximum temperature (50 °C) was reached, the stimulation was stopped and the maximum temperature was

recorded as the threshold value. In this study, all participants (and at all time points) pressed the button to indicate their detection or pain threshold; the maximum temperature of 50°C was never reached.

The pain induced by graded thermal stimuli was assessed with a 100-mm visual analog scale (VAS). All participants received stimulation with three pseudorandomized heat stimuli (42 °C, 44 °C and 46 °C). For each stimulus, participants were asked to rate the intensity of the pain on a VAS, extending from “no pain” to “maximal imaginable pain”. For each stimulation, the thermode temperature gradually rose from baseline temperature (32 °C) at a rate of 1 °C/s. Once the target temperature was reached, it was maintained for 2 s and the temperature then returned to baseline. Stimuli were separated by an interval of at least 45 s. Pain sensitization was prevented by applying the thermode to adjacent regions of the forearm, never using the same site for consecutive stimuli.

For more precise assessments of pain sensitivity than could be achieved with the responses to arbitrary temperatures, intensity response curves were calculated (Figure 3). This is a better approach to the assessment of sensitivity, as it can be used to determine the half maximal effective temperature, or ET₅₀, corresponding to the stimulation temperature required to induce 50 % of the maximal response (pain intensity of 5/10).

The data were modeled with a sigmoidal function:

$$f(\text{temperature}) = \text{max} + \frac{\text{min} - \text{max}}{1 + \left(\frac{\text{temperature}}{\text{ET}_{50}}\right)^{\text{hillslope}}}$$

As the VAS is a bounded scale, minimum (min) and maximum (max) pain scores were set at 0 and 10, respectively. Hillslope, the slope of the curve, and ET₅₀ were left free. Temperatures were expressed on a logarithm scale. The statistical power of the modeling approach was increased by calculating sigmoidal fits over 4-hour time epochs, corresponding to two evaluations of pain sensitivity for each of the three stimuli (42 °C, 44 °C and 46 °C), providing six points on the regression curve

(Supplementary Figure 1). The ET_{50} values were extracted from each of the nine sigmoidal regressions (see formula above; Figure 3A) and plotted over time (Figure 3B).

Body temperature

Core body temperature was measured every 2 h, with an ear thermometer (Braun ThermoScan Pro 6000, Welch Allyn, New York, USA). Body temperature was measured within 2-3 seconds, with a precision of 0.2 °C.

Electrocardiogram

An electrocardiogram (ECG) was recorded with two adhesive skin electrodes (BlueSensor N, Ambu, Ballerup, Denmark) positioned on the sternum and the lateral thorax (RA, LL, respectively, Fontaine bipolar precordial leads). The signal was recorded at 256 Hz, with a Vitaport 4 digital recorder (Temec Instruments, Kerkrade, The Netherlands), to assess autonomic nervous system activity. Heart rate (HR) and heart-rate variability (HRV) were analyzed on the basis of the bipolar ECG signal. R-wave peak detection was performed over 10-second windows during a 4.5-minute baseline resting episode. For interval analysis, data were resampled at a rate of 10 Hz. RMSSD was determined to estimate the vagally mediated changes reflected in HRV^{29,30}. It was not possible to obtain ECG data for the first participant, for technical reasons, so ECG analysis was performed for 11 participants.

Melatonin

Saliva was collected hourly, with cotton swabs placed directly in the mouth of the participant (Salivettes, Sarstedt, Nümbrecht, Germany). Samples were stored at -20 °C until centrifugation and assay. Melatonin levels were measured with an in-house radioimmunoassay ¹²⁵I (RIA). This assay was based on a competition technique. The radioactive signal, reflecting the amount of ¹²⁵I-labeled

melatonin, was therefore inversely proportional to the concentration of melatonin in the sample. The sensitivity of the assay was 1.5 pg/mL. The inter-assay coefficients of variation for high (18.5 pg/mL) and low (10 pg/mL) melatonin-concentration controls were 19 % and 22 % respectively, and the mean intra-assay coefficient of variation was below 10 %. We determined the circadian melatonin profile of each participant over a 24-hour day, by applying a three-harmonic regression individually to the raw data collected during the CR (days 2 and 3)^{31,32}. The model equation was:

$$f(\text{time}) = \text{mesor} + \text{amplitude1} \times \cos\left(2\pi \times \frac{\text{time}}{\text{tau}} + \text{phase1}\right) + \text{amplitude2} \times \cos\left(2\pi \times \frac{\text{time}}{\text{tau}} + \text{phase2}\right) + \text{amplitude3} \times \cos\left(2\pi \times \frac{\text{time}}{\text{tau}} + \text{phase3}\right)$$

In the model, Tau (the circadian period) was constrained between 23.5 and 24.5 h; mesor, amplitudes (1 to 3) and phases (1 to 3) were set free.

The dim light melatonin onset (DLMO_n), corresponding to the circadian phase, was calculated for each participant. DLMO_n was defined as the time at which the ascending phase of the melatonin profile crossed the 25 % threshold of the peak-to-trough amplitude of the fitted curve. Due to technical problems with some saliva samples, the full 24-hour melatonin profile could not be obtained for two participants. For one of these participants, DLMO_n was calculated on the basis of melatonin levels during the habituation day (day 1), rather than during the CR, for which we could not determine melatonin concentrations. For the second participant, in the absence of melatonin concentration data (flat profile below the limit of quantification of the assay), DLMO_n was estimated from the mean phase angle calculated between habitual bedtime and DLMO_n (calculated from data published by Gronfier et al., 2004)³¹. The average DLMO_n was 21:36 ± 01:08 in our group of subjects.

Statistics

Outliers were identified on the basis of individual normalized data (*z*-scores) and were excluded from subsequent analyses (outlier.test, R, Version 3.6.1 - 2019-07-05, R Foundation for Statistical Computing, Vienna, Austria). We reduced inter-individual variability, by normalizing all subjective data by calculating individual *z*-scores and smoothing them with a moving average (calculated on 3 points). The endogenous circadian phase was taken into account for each participant, by aligning the data with the onset of melatonin secretion (DLMO_n). As DLMO_n occurred at different times in different participants, individual melatonin onset values were set to 0 (DLMO_n = circadian time 0), and all measurement times are expressed relative to melatonin onset. We modeled the effects of time on the responses observed during the 34-hour constant routine, using an additive model including a linear component (homeostatic, process S) and a sinusoidal component (circadian, process C). The equation of the combined model was:

$$f(\text{time}) = y_0 + a \times \text{time} + \text{mesor} + \text{amplitude} \times \cos\left(2\pi \times \frac{\text{time}}{\text{tau}} + \text{phase}\right)$$

Tau (circadian period) was constrained between 23.5 and 24.5 hours^{33,34}, whereas all other parameters were left free. Once the parameters of the combined model had been defined, process S and process C were modeled separately. The homeostatic component (process S) was regressed against the linear component of the model: $f(\text{time}) = y_0 + a \times \text{time}$. The circadian rhythmicity (process C) of the data was regressed against the sinusoidal component of the model: $f(\text{time}) = \text{mesor} + \text{amplitude} \times \cos\left(2\pi \times \frac{\text{time}}{\text{tau}} + \text{phase}\right)$.

Statistics were calculated with R (Version 3.6.1 - 2019-07-05, R Foundation for Statistical Computing, Vienna, Austria). Results were considered significant if $p < 0.05$. Results are expressed as mean \pm SD when reporting participant demographics/anthropometrics in the methods sections and as mean \pm SEM for data presented in the results section (unless stated otherwise).

Data Availability

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

Results

Pain rhythmicity is regulated by homeostatic and circadian processes

Twelve healthy men aged 22.7 ± 3.3 years (mean \pm SD) participated in a 56-hour experimental protocol (Figure 1) including a 34-hour highly controlled constant routine (CR) designed to unmask endogenous rhythmicity (enforced wakefulness, constant posture, low physical and cognitive activity, constant dim light, equicaloric snacks every hour)²¹. We assessed the effect of time-of-day on pain sensitivity, by measuring heat pain every two hours during the 34 h of constant routine. In accordance with the current view that two main processes regulate sleep³⁵, and in agreement with studies showing that subjective sleepiness^{13,36} and physiological functions (such as executive functions³⁷) and cortical brain responses (measured by EEG¹⁴ and fMRI⁸) are influenced by both sleep pressure and the circadian timing system, we then modeled the effect of time on pain with an additive mathematical model including a linear component (sleep-related homeostatic drive - process S) and a sinusoidal component (circadian drive - process C)¹³.

Pain sensitivity increases with sleep debt

We probed subjective pain (visual analog scale ratings) in response to two-second heat stimuli (42 °C, 44 °C and 46 °C) every two hours over the entire 34-hour constant routine (Figure 2A, 2B, 2C and Supplementary Figure 2; all $R^2 > 0.72$). A linear component was observed for the stimuli at 44 °C and 46 °C (Figure 2E and 2F; all $p < 0.0001$; all $R^2 > 0.73$), but not for the less painful stimuli at 42 °C (Figure 2D; $p = 0.23$; $R^2 = 0.10$). Our results thus confirm the known relationship between sleep deprivation and greater pain sensitivity³⁸⁻⁴⁰, but suggest that this relationship may not apply to low

levels of pain. As the participants were in a constant state of wakefulness during the CR, the linear component of our model translates the effect of sleep debt and reflects homeostatic sleep pressure. The slope of the linear regression line increased with stimulation temperature (Figure 2D, 2E, and 2F), so the largest changes in amplitude were observed for stimuli at 46 °C, which caused a change in pain level of 1/10 on the visual analog scale (VAS). As pain responses were measured at three arbitrary temperatures, we then used a modeling approach (classically used in pharmacology and photobiology^{41,42} to extract an overall pain sensitivity value (Figure 3). The mathematically modelled sigmoidal intensity response curve (based on the combined results obtained at 42, 44 and 46 °C) yielded sensitivity values (ET₅₀) that confirmed the results reported above; a linear increase in sensitivity to pain with time awake (lower ET₅₀ values) (Figure 3C; R² = 0.81; *p* < 0.01).

Pain sensitivity is driven by the circadian timing system, with maximal pain experienced during the night

Subjective measurements of pain in response to two-second thermal stimuli (42 °C, 44 °C and 46 °C) revealed that pain sensitivity was influenced not only by sleep pressure, but also by the circadian timing system (Figure 2A, 2B and 2C; all R² > 0.72). Indeed, independently of the effect of sleep pressure, a sinusoidal component in our model strongly accounted for changes in pain sensitivity across the 34 hours of constant routine, with a pain sensitivity peak between 3:00 and 4:30 for both the responses to graded stimuli (Figure 2G, 2H and 2I) and heat pain thresholds obtained using stimulation ramps (Supplementary Figure 3C). These results were confirmed by the modeling of a sigmoidal intensity response curve, which also showed a strong circadian rhythmicity of pain sensitivity (Figure 3D; R² = 0.93) and a pain peak in the middle of the night (at 4:30). Interestingly, the lack of circadian rhythmicity for warm non-painful stimuli (Supplementary Figure 4C; R² = 0.13) suggests that the rhythmicity of pain sensitivity is specific to pain and is not related to a general rhythmicity of thermal sensitivity.

Changes in pain sensitivity over the 24-hour day are primarily induced by the circadian timing system rather than by sleep pressure

We investigated the relative contributions of sleep pressure and circadian drives to pain sensitivity, by calculating the mean changes in both these components and expressing them relatively to the total amplitude over 24 hours (Figure 2 and Supplementary Figure 5). Using the 24h profile at 44°C, we find that the amplitude of pain sensitivity is 0.83 (Fig. 2B, combined model, nadir of -0.40 at circadian time [CT] -6.5 and acrophase of +0.44 at circadian time 6.5), of which 0.18 are due to the homeostatic trend (from -0.12 at CT -6.5 to +0.06 at CT 6.5), and 0.65 to the circadian oscillation (from -0.27 at CT -6.5 to +0.38 at CT 6.5). This reveals that the circadian system accounted for ~80 % ($0.65/0.83 = 78\%$) of the full magnitude of pain sensitivity changes over 24 hours, the remaining ~20 % ($0.18/0.83 = 22\%$) being accounted for by the homeostatic component.

Phase relationships between the circadian components of pain modulation and interoceptive responses

Having identified a circadian drive for pain, we investigated whether the rhythm of pain sensitivity displayed phase relationships with interoceptive responses. Using cross-correlation analyzes, we identified a clear phase opposition (~12-h lag) between the rhythms of pain sensitivity and core body temperature (Figure 4A; Supplementary Figure 6), with the acrophase of pain (at 3:30, 95% confidence interval [CI] for phase= [2:45,4:15], 95% CI for amplitude = [0.52, 0.80]) occurring at about the same time as the nadir of core body temperature (at 3:00, 95% CI for phase= [2:00, 3:30], 95% CI for amplitude = [1.56, 1.95]). We also found that pain sensitivity peaked 1.5 hours after endogenous melatonin secretion (at 2:00, 95% CI for fundamental phase= [1:44, 2:16], 95% CI for fundamental amplitude = [21.0, 24.0]) (Figure 4B). Autonomic nervous system responses displayed strong circadian

rhythmicity, with a nadir of vagal activity (minimal heart rate) at 2:00 (95% CI for phase= [1:44, 2:16], 95% CI for amplitude = [1.42, 1.64]) and a peak of parasympathetic activity (maximal RMSSD) also at 2:00 (95% CI for phase= [1:10, 2:50], 95% CI for amplitude = [0.70, 1.10]), preceding the pain sensitivity peak by 1.5 hours (Figure 4C and 4D).

Discussion

This is the first highly controlled laboratory study specifically designed to investigate pain rhythmicity and its underlying driving mechanisms in healthy individuals. Our results unequivocally demonstrate that pain sensitivity is endogenously driven by the circadian timing system, and that sleep and sleep deprivation have a much weaker influence on pain sensitivity than previously thought.

A limited number of previous studies have systematically investigated the rhythmicity of pain perception in healthy individuals. Careful analysis reveals that published results are equivocal, some studies showing no rhythmicity, others reporting maximal sensitivity either during the day or during the night¹⁵⁻¹⁹. A recent modelling work, using pooled datasets from four experimental studies, proposed a sinusoidal model of pain sensitivity very similar to ours, with a peak sensitivity close to midnight⁴³. However, because the model was built on data obtained from different populations and protocols, and collected during either sleep, wake, rest, activity, light, or dark conditions, both the phase (timing) and the origin of this rhythmicity in pain sensitivity cannot be attributed to any underlying timing mechanism, neither circadian, nor sleep-related. Overall, although often claimed by the authors, none of the previous studies has demonstrated that pain perception was circadian, i.e. of endogenous origin.

By contrast, our results, showing a strong sinusoidal oscillation of pain sensitivity in a constant routine protocol, i.e. in the absence of rhythmic influences and times cues, provide unequivocal evidence that the rhythmicity of pain sensitivity is driven from within, by the endogenous circadian timing system, and does not result from influences evoked the light-dark cycle, the rest-activity cycle, or the sleep-

wake cycle. Indeed, if pain sensitivity were to be regulated exclusively by the sleep/wake cycle, as previously thought, we would have observed a peak in pain sensitivity at the end of our 34-hour experimental constant-routine day and not in the middle of it (after 20 hours) as we did. The very observation that the cyclicity of pain sensitivity is driven by the circadian system, independently from the sleep/wake cycle or any other environmental cycle, demonstrates that both the rhythmicity and its specific timing (its phase) must be fundamental physiological needs in humans. Contrarily to the widely held view that pain sensitivity is driven by the sleep-wake cycle (decreasing during sleep and increasing during the day), our quantification that the circadian oscillation accounts for ~80% of the full magnitude of pain sensitivity over the 24-h, and that sleep deprivation accounts for only ~20% of it, reveals that sleep pressure has in fact a very modest effect on pain in healthy young individuals.

The pathways linking the circadian timekeeping system to pain perception cannot be inferred from this study, but the suprachiasmatic nucleus is undoubtedly the starting point, and the subcortical and cortical regions of the brain (e.g. somatosensory cortices, insula, thalamus, prefrontal cortex), often referred to as the “pain matrix”⁷ are likely to be involved, given that they have been shown to be regulated by the sleep/wake cycle or the circadian clock⁸⁻¹⁰. Our study suggests an interoceptive regulation of pain, where the circadian pacemaker is likely to be central. Pain is traditionally regarded as an exteroceptive response depending on both the somatosensory and emotional systems, however, it has been suggested that it may also be part of the interoceptive system, relating to the condition of the body^{6,44}. The interoceptive responses underlying the maintenance of the internal environment of the body are organized in a hierarchical manner. They involve a number of extensively connected physiological systems, so any change in one interoceptive function is usually associated with changes in one or several other interoceptive functions.

Our data are consistent with this view as they show that, like other interoceptive functions, pain is driven by a time-specific circadian rhythm that is directly related to the rhythmicity of other functions. The phase opposition we find between pain sensitivity and core body temperature (CBT) suggests an

interaction between thermoregulation and nociception ⁴⁵, both of which are components of the interoceptive system ^{6,44}. The phase relationships observed between the rhythms of the sympathetic and parasympathetic systems (as assessed by cardiovascular measurements) and pain are also consistent with this hypothesis and suggest the existence of strong interactions between the nociceptive pathways and the autonomic nervous system (ANS) ⁴⁶⁻⁴⁸. The circadian timing system may, via the SCN, serve as a key interface between pain and other interoceptive functions. The mechanisms underlying these interactions are unclear. Interestingly, our data suggest that they are probably not mediated by melatonin, a nocturnal hormone released by the pineal gland, although exogenous administration is generally reported to induce antinociceptive effects ⁴⁹⁻⁵¹. Such effects are not consistent with the temporal relationship between peak pain sensitivity and peak endogenous melatonin secretion reported here, which instead suggests a pronociceptive effect of melatonin. None of these mechanisms can be validated on the basis of our results as we describe only temporal relationships between time series, but they could all be relatively easily tested experimentally to determine their causality.

Alternatively, the circadian rhythmicity of pain may be accounted for by direct control of the nociceptive network (or the cognitive/emotional structures) by the SCN. In this regulatory model of pain regulation, the circadian system may be responsible for controlling the precise timing of nociception ⁶. As the thalamus is a key player in the nociceptive pathway and projections from the SCN to the anterior paraventricular thalamus have been identified ⁵², pain sensitivity may be directly modulated by this brain structure over the course of the 24-hour day.

Multiple other pathways could be involved. Using the same highly controlled experimental conditions we employed here, a study showed that ~15% of all identified metabolites in plasma and saliva are under circadian control in humans ⁵³. These include metabolites involved in pain pathways, and recently identified metabolites of neuroinflammation specifically found elevated in patients with neuropathic pain compared to those without neuropathic pain ⁵⁴. Whether those metabolites are

involved in all clinical conditions of pain or in experimentally induced pain is unknown, but overlapping the human circadian metabolome and our results allows to propose that the circadian system regulates pain sensitivity through multiple pathways, both in normal and pathological situations. The influence of sleep and sleep deprivation on pain sensitivity is modest in terms of its impact on the full magnitude of pain sensitivity over the 24-h, but it is not negligible. The linear increase in pain sensitivity that we find during enforced wakefulness, after mathematically removing the circadian component, confirms that pain sensitivity does increase with time spent awake and reveals that it is under the influence of an independent (from the circadian system) homeostatic drive, possibly related to that involved in the buildup of sleep pressure from waketime to bedtime³⁵. This finding is consistent with the studies we previously discussed^{15-19,54} and with the classically described interaction between pain and sleep^{1,55-58}, whereby pain sensitivity appears to be driven by the sleep/wake cycle, with pain perception low in the morning after a night of good-quality sleep, increasing during the day to reach a peak before bedtime, and then decreasing during sleep⁴⁰. In the absence of sleep (after one night of total sleep deprivation), pain sensitivity has been shown to be higher than it was at the same time on the previous day^{38,39}, highlighting that there is an analgesic effect of sleep and/or a hyperalgesic effect of sleep deprivation. This sleep drive is usually considered to explain why sleep disorders, such as insomnia, are associated with an exacerbation of clinical pain^{40,59}. The reciprocal interactions between sleep homeostasis and pain may result from functional changes in the interconnected sleep and pain systems. Consistent with this hypothesis, sleep loss is associated with an increase in the activation of somatosensory brain areas induced by painful stimuli, potentially reflecting an amplification of neuronal responses in the cortical nociceptive systems and/or a disinhibition of normal thalamocortical pain signaling⁵⁸. In addition, sleep deprivation blunts activity in areas of the brain involved in endogenous pain modulation, such as the striatum and insular cortex⁵⁸. The specific mechanisms underlying the interactions between pain and sleep remain unknown, but may involve sleep-promoting factors, such as adenosine⁶⁰. Adenosine accumulates with increasing homeostatic sleep pressure

during wakefulness, reaching high levels at the end of the day ^{61,62}, and then declining during sleep ⁶³. In addition to its role in the sleep/wake cycle, adenosine is also involved in the nociceptive system and may play an anti- or pronociceptive role, depending on the receptors activated ^{64,65}. Thus, the hyperalgesic effect of constant wakefulness reported here may be at least partly due to adenosine accumulation, leading to A1B receptor activation ⁶². Obviously, other mediators, such as cytokines, which also play a role in both pain ⁶⁶ and sleep regulation ⁶⁷, may be involved in the sleep-related modulation of pain sensitivity.

This study has a number of limitations. First, our protocol was conducted under non-ecological and highly controlled laboratory conditions, which were nevertheless absolutely essential to dissect out the rhythmic and endogenous elements of pain sensitivity. Pain sensation may be different in real-life conditions, but the endogenous mechanisms controlling pain sensitivity are expected to be the same. The modest influence of sleep deprivation on pain sensitivity suggested by our model, may also be different in real-life conditions. Indeed, prior to their experimental session in the laboratory, our participants underwent 3 weeks of sleep monitoring, during which time they slept on average 8 hours per night, and ensured they were sleep satiated upon arrival. In real life conditions, where sleep deprivation is common in our societies, the strength of sleep-related drive may be higher than in our conditions. This does not invalidate our model, but asks for its careful interpretation in different conditions ⁶⁸ and also for its evaluation in conditions of sleep deprivation. Second, pain intensity was evaluated in healthy participants, with an experimental heat pain paradigm. It is conceivable that sleep pressure and the circadian timing system have the same effect on any type of pain, but our results cannot be directly extrapolated to other pain modalities or to clinical populations (both patients suffering from pain disorders, and patients suffering sleep and/or circadian disorders). Third, the population examined in this study consisted exclusively of men. Circadian physiology is very similar in men and women, with only minor differences, such as a slightly larger amplitude ^{69,70}, and a slightly shorter period ³³ in women, but our results should not be extrapolated to premenopausal women, in

whom the menstrual cycle may modulate both the homeostatic and circadian drives of pain sensitivity. Although sex differences in pain have also been reported, we do not expect that women would show different circadian or homeostatic profiles for pain sensitivity. Nonetheless, studies in women are warranted.

In conclusion, our results reveal the neurobiological mechanisms driving the rhythmicity of pain perception in humans, with the main driving brain structure located in the suprachiasmatic nuclei of the hypothalamus. We show that pain sensitivity is controlled by two superimposed processes: a strong circadian component and a modest homeostatic sleep-related component. This finding may have clinical implications, as dysregulations of the circadian system have been implicated in a number of diseases with major consequences for health ¹¹. Such alterations may also be involved in the pathophysiology of some chronic pain syndromes, as suggested for cluster headaches, for example ⁷¹. The existence of a circadian rhythmicity in pain suggests that the efficacy of pain management could be optimized using circadian medicine ^{72,73}. With this approach, analgesic treatments could be administered according to the each patient's internal time (circadian time) rather than according to a uniform timing schedule mostly based on pragmatic considerations ⁷⁴⁻⁷⁶. Such circadian approaches have already proved effective in cancer treatment ⁷⁷, but have not been systematically evaluated for the treatment of pain. Individually timed medication could improve chronic pain management and greatly improve patients' quality of life, not only by improving treatment efficacy but also by reducing the adverse effects of painkillers (including those pejorative to sleep and circadian physiology).

Author contributions

The experiment was conceived by CG, and designed by CG, DB and ID. Data were collected by ID and CG. Melatonin assays were conducted by VR. Data were analyzed and interpreted by ID and CG. ID, CG and DB wrote the manuscript, and VR revised the manuscript. All authors agree to be accountable for all aspects of the work.

Acknowledgments

We wish to thank all the volunteers who participated in this study. We also wish to thank the staff and students, and especially Pauline Kirchhoff who participated in data collection and analysis. Special thanks also go to Dr Alain Nicolas, who conducted the medical and physical examinations.

Funding

This work was supported by fundings from “Société Française de Recherche et Médecine du Sommeil” (SFRMS) and “Société Française d’Etude et de Traitement de la Douleur” (SFETD) to ID, and grants from the French National Research Agency (ANR-12-TECS-0013-01 and ANR-16-IDEX-0005) to CG. ID was supported by a doctoral fellowship from the French “Ministère de l’Enseignement Supérieur et de la Recherche”.

Competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

Supplementary material will be / is available at *Brain* online, and is attached at the end of this preprint version.

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Figure legends

Figure 1. Overview of the experimental protocol. After a day of habituation (day 1) and an 8-h sleep episode, participants were subjected to a 34-hour constant routine (CR: days 2 and 3). Melatonin levels were assessed hourly (blue stars); pain sensitivity, temperature, heart rate and RMSSD were evaluated every two hours (red circles). Participants arrived at about 10:00 on day 1 (down arrow) and left the laboratory at about 18:00 on day 3 (up arrow). Gray rectangles represent wakefulness in dim light (~0.5 lux) and black rectangles represent scheduled sleep in darkness.

Figure 2. Mean pain intensities in response to 2-second heat stimuli at 42 °C, 44 °C and 46 °C are rhythmic across the 34-h constant routine protocol ($n = 12$). Dark bars correspond to the average timing of habitual sleep episodes (biological night). Circadian time 0 corresponds to dim light melatonin onset (DLMon, mean $\approx 21:30$). (A - C) Combined models (sum of linear and sinusoidal components) applied to normalized data (mean \pm SEM) for stimuli at 42 °C (A. $R^2 = 0.72$), 44 °C (B. $R^2 = 0.92$) and 46 °C (C. $R^2 = 0.92$). (D - F) Linear components for stimuli at 42 °C (D. $R^2 = 0.10$; $p = 0.23$), 44 °C (E. $R^2 = 0.73$; $p < 0.0001$) and 46 °C (F. $R^2 = 0.81$; $p < 0.00001$). Pain sensitivity

increases with time spent awake for stimuli at 44 °C and 46 °C. (**G - I**) Sinusoidal components for stimuli at 42 °C (**G**. $R^2 = 0.70$), 44 °C (**H**. $R^2 = 0.90$) and 46 °C (**I**. $R^2 = 0.86$). Pain sensitivity follows a circadian rhythm, with maximal pain at 3:30 (42 °C and 44 °C) or 3:00 (46 °C).

Figure 3. Mean pain sensitivity (ET_{50}) is rhythmic across the 34-h constant routine protocol (n = 12). (**A**) Intensity response curves calculated on the 6 measures obtained at 42, 44, and 46 °C over two consecutive 2-hour segments (9 curves; all R^2 between 0.68 and 0.99). Y axis expresses the decimal logarithm of the stimulation temperature. (**B**) Combined model (sum of linear and sinusoidal components) applied to raw ET_{50} values ($R^2 = 0.96$). (**C**) Linear component ($R^2 = 0.81$; $p < 0.01$). ET_{50} s decrease and pain sensitivity increases with time spent awake. (**D**) Sinusoidal component ($R^2 = 0.93$). Pain sensitivity follows a circadian rhythm with maximal pain at 4:30. (**B**), (**C**) and (**D**). Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO_n (mean DLMO_n \approx 21:30). Right X axis has been adapted from left X axis to express ET_{50} directly in °C and not in log °C.

Figure 4. Phase relationships between circadian components of pain sensitivity and temperature (A), melatonin (B), heart rate (C) and parasympathetic activity (D) across the 34-hour constant routine protocol. Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO_n (mean DLMO_n \approx 21:30). All curves represent the sine component of the modeled parameter. **All panels.** Circadian rhythm of VAS pain intensity scores in response to a two-second stimulation at 44 °C, with a sensitivity peak at 3:30 (green curve; $R^2 = 0.90$). (**A**) Circadian rhythm of baseline body temperature, with a minimal core body temperature at 3:00 (blue curve; $R^2 = 0.97$). (**B**) Circadian rhythm of melatonin secretion (pg/mL), with a secretion peak at 2:00 (yellow curve; $R^2 = 0.98$). (**C**) Circadian rhythm of heart rate, with a minimal heart rate at 2:00 (red curve; $R^2 = 0.99$). (**D**) Circadian rhythm of RMSDD (parasympathetic activity), with an activity peak at 2:00 (purple curve; $R^2 = 0.89$).

Figures

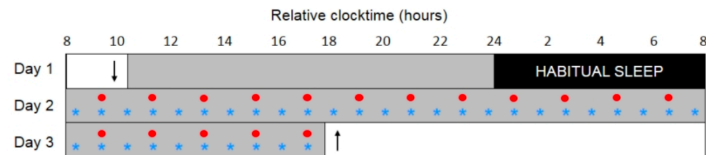


Figure 1. Overview of the experimental protocol. After a day of habituation (day 1) and an 8-h sleep episode, participants were subjected to a 34-hour constant routine (CR: days 2 and 3). Melatonin levels were assessed hourly (blue stars); pain sensitivity, temperature, heart rate and RMSSD were evaluated every two hours (red circles). Participants arrived at about 10:00 on day 1 (down arrow) and left the laboratory at about 18:00 on day 3 (up arrow). Gray rectangles represent wakefulness in dim light (~ 0.5 lux) and black rectangles represent scheduled sleep in darkness.

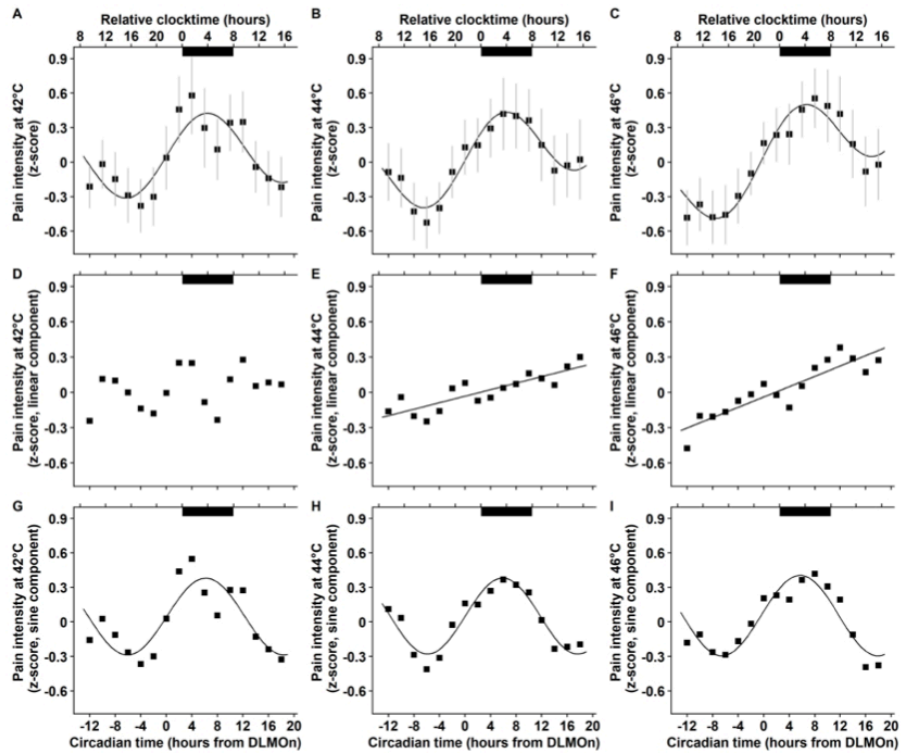


Figure 2. Mean pain intensities in response to 2-second heat stimuli at 42 °C, 44 °C and 46 °C are rhythmic across the 34-h constant routine protocol ($n = 12$). Dark bars correspond to the average timing of habitual sleep episodes (biological night). Circadian time 0 corresponds to dim light melatonin onset (DLMO), mean $\approx 21:30$). (A - C) Combined models (sum of linear and sinusoidal components) applied to normalized data (mean \pm SEM) for stimuli at 42 °C (A. $R^2 = 0.72$), 44 °C (B. $R^2 = 0.92$) and 46 °C (C. $R^2 = 0.92$). (D - F) Linear components for stimuli at 42 °C (D. $R^2 = 0.10$; $p = 0.23$), 44 °C (E. $R^2 = 0.73$; $p < 0.0001$) and 46 °C (F. $R^2 = 0.81$; $p < 0.00001$). Pain sensitivity increases with time spent awake for stimuli at 44 °C and 46 °C. (G - I) Sinusoidal components for stimuli at 42 °C (G. $R^2 = 0.70$), 44 °C (H. $R^2 = 0.90$) and 46 °C (I. $R^2 = 0.86$). Pain sensitivity follows a circadian rhythm, with maximal pain at 3:30 (42 °C and 44 °C) or 3:00 (46 °C).

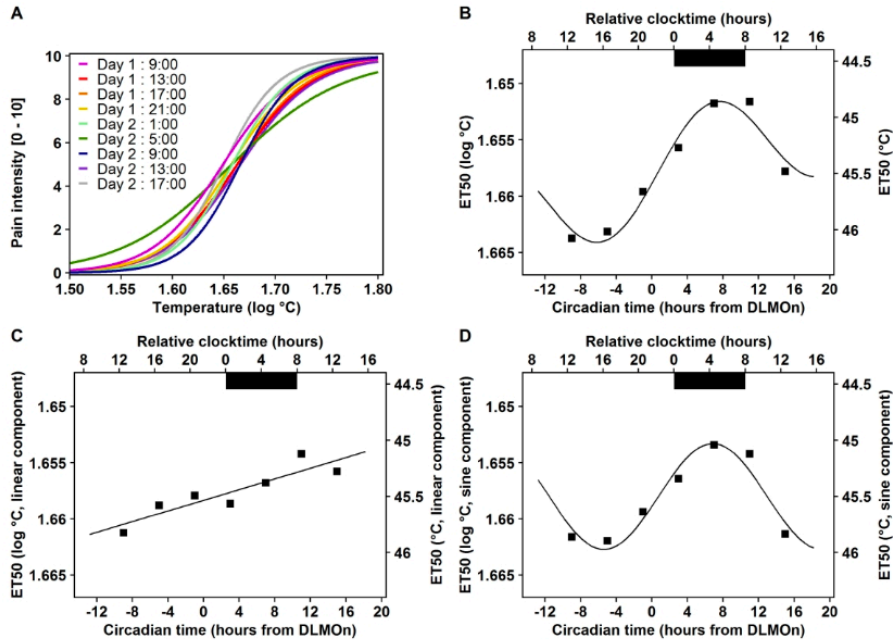


Figure 3. Mean pain sensitivity (ET50) is rhythmic across the 34-h constant routine protocol ($n = 12$). (A) Intensity response curves calculated on the 6 measures obtained at 42, 44, and 46 °C over two consecutive 2-hour segments (9 curves; all R^2 between 0.68 and 0.99). Y axis expresses the decimal logarithm of the stimulation temperature. (B) Combined model (sum of linear and sinusoidal components) applied to raw ET50 values ($R^2 = 0.96$). (C) Linear component ($R^2 = 0.81$; $p < 0.01$). ET50s decrease and pain sensitivity increases with time spent awake. (D) Sinusoidal component ($R^2 = 0.93$). Pain sensitivity follows a circadian rhythm with maximal pain at 4:30. (B), (C) and (D). Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO (mean DLMO $\approx 21:30$). Right X axis has been adapted from left X axis to express ET50 directly in °C and not in log °C.

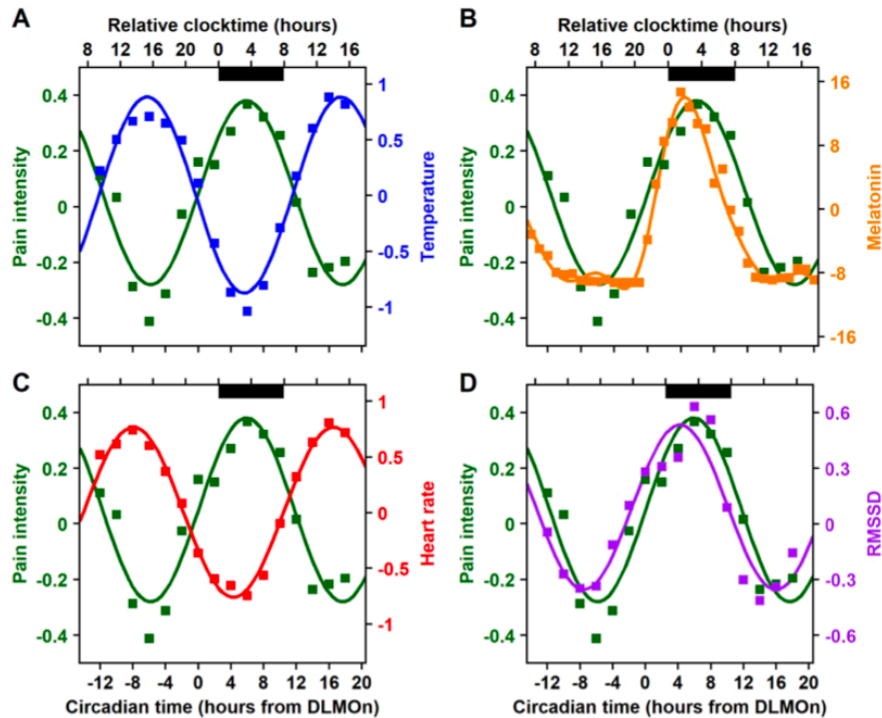
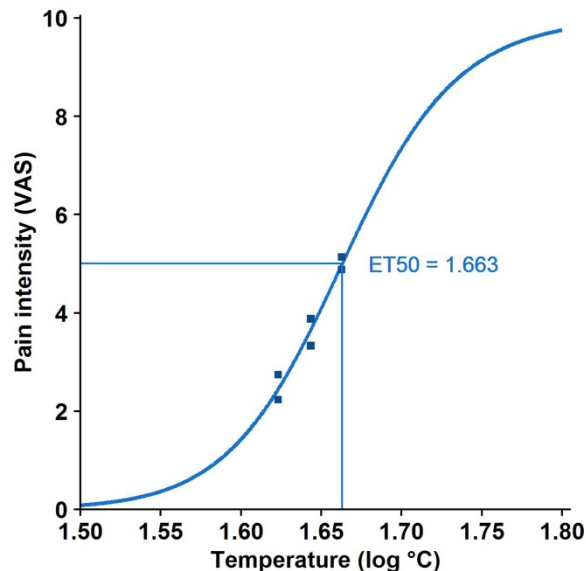


Figure 4. Phase relationships between circadian components of pain sensitivity and temperature (A), melatonin (B), heart rate (C) and parasympathetic activity (D) across the 34-hour constant routine protocol. Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO (mean DLMO \approx 21:30). All curves represent the sine component of the modeled parameter. All panels. Circadian rhythm of VAS pain intensity scores in response to a two-second stimulation at 44 °C, with a sensitivity peak at 3:30 (green curve; $R^2 = 0.90$). (A) Circadian rhythm of baseline body temperature, with a minimal core body temperature at 3:00 (blue curve; $R^2 = 0.97$). (B) Circadian rhythm of melatonin secretion (pg/mL), with a secretion peak at 2:00 (yellow curve; $R^2 = 0.98$). (C) Circadian rhythm of heart rate, with a minimal heart rate at 2:00 (red curve; $R^2 = 0.99$). (D) Circadian rhythm of RMSSD (parasympathetic activity), with an activity peak at 2:00 (purple curve; $R^2 = 0.89$).

Circadian rhythmicity of pain sensitivity in humans

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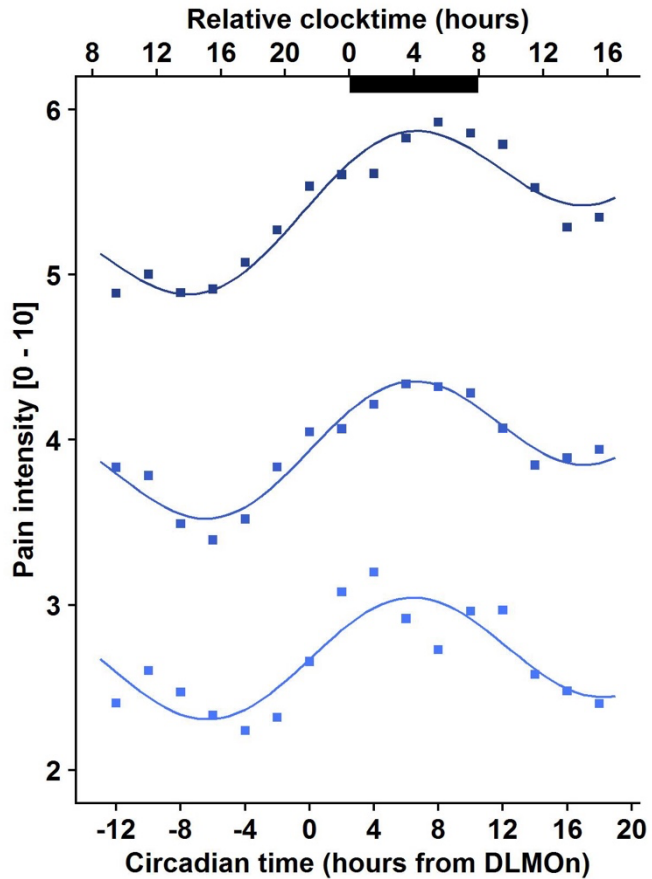
Supplementary Information



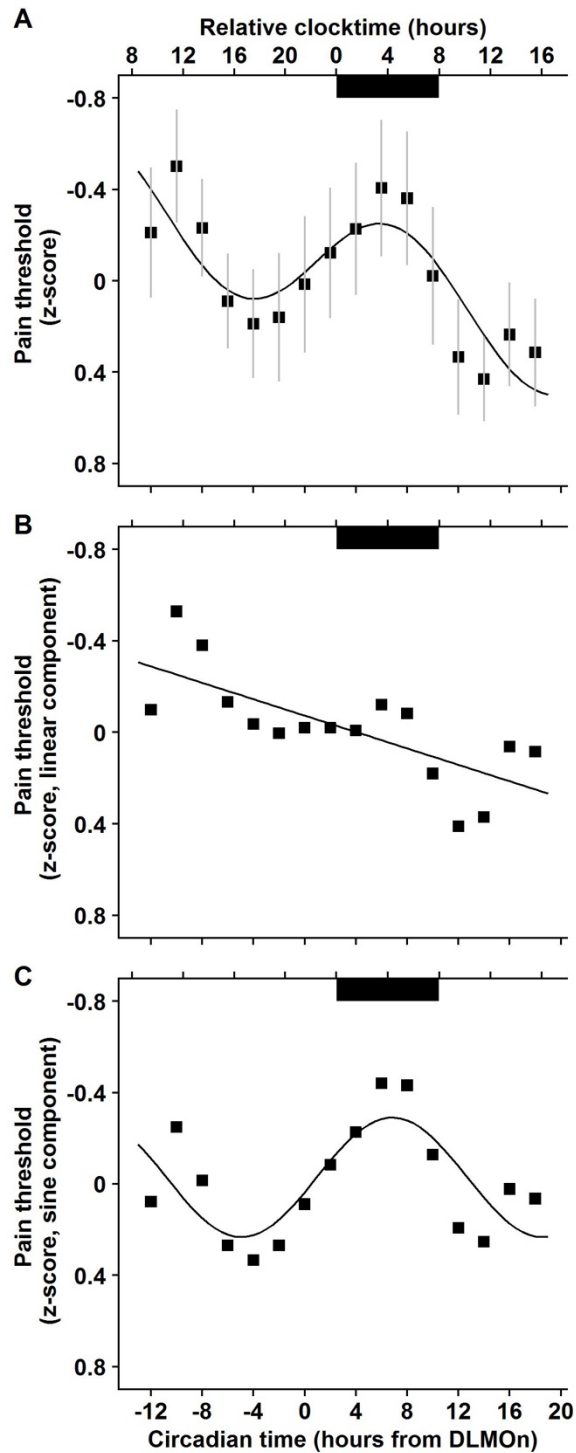
Supplementary Figure 1. Pain Intensity Response Curve (IRC) at circadian time -9 (~ 12:30). Sigmoidal regression, calculated on a 4-hour time epoch (10:30 – 14:30), corresponding to 2 evaluations of pain sensitivity to each of the 3 heat stimulations (42 °C, 44 °C and 46 °C), providing 6 points for the regression. The ET_{50} value, corresponding to a stimulus inducing a pain of 5/10, is extracted from the sigmoidal regression (here $ET_{50} = 1.66 \log[\text{temperature}]$).

Time-of-day mechanisms of heat detection and pain thresholds

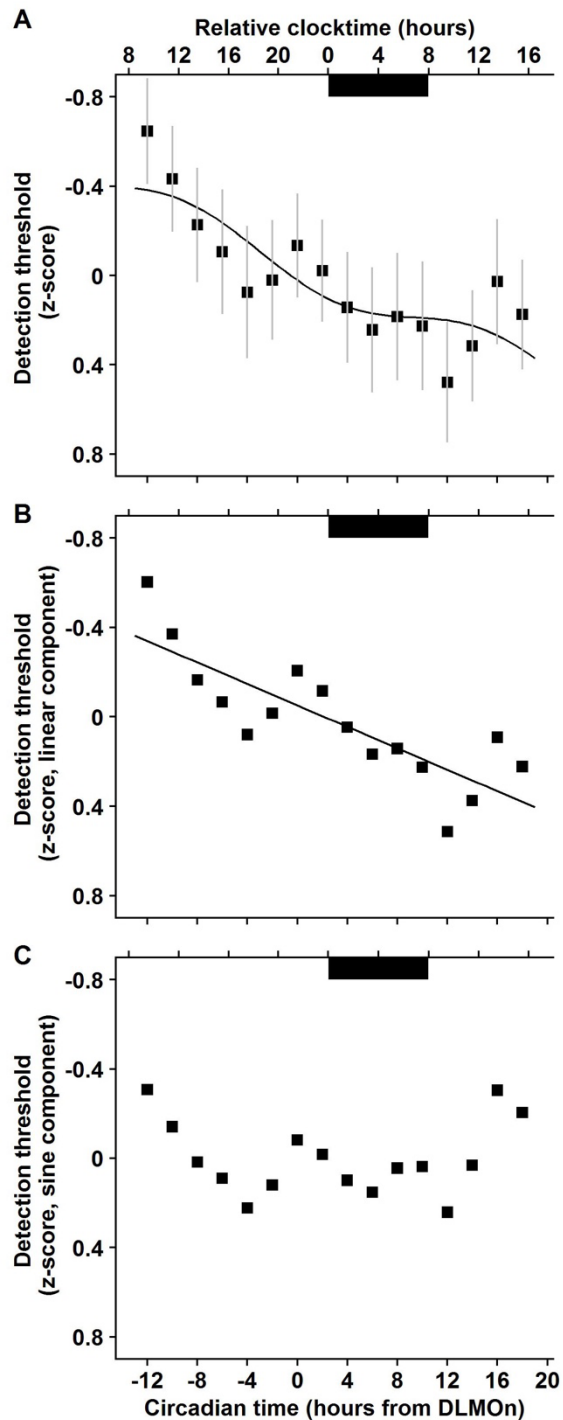
Heat pain thresholds and warm detection thresholds were measured every 2 hours throughout the whole 34-hour constant routine (Supplementary Figures 2 and 3; all $R^2 > 0.69$). The significant linear trend observed for heat pain thresholds (Supplementary Figure 2; $p < 0.01$; $R^2 = 0.53$), suggests a decrease in pain sensitivity with sleep pressure. This result might reflect a deterioration of cognitive-motor performances (slower reaction times) associated with sleep pressure (1,2). The fact that this effect was not specific to the heat pain threshold, since a similar relationship was seen for the warm detection threshold (Supplementary Figure 3; $R^2 = 0.68$; $p < 0.0001$), is consistent with this hypothesis. The strong circadian rhythm of pain sensitivity (with a peak at 4:30), assessed through heat pain threshold measures, confirms the results found with graded heat stimuli (and presented in the main article). The lack of circadian rhythmicity for warm non-painful stimuli (Supplementary Figure 4C; $R^2 = 0.13$) suggests that the rhythmicity of pain sensitivity is specific to pain and is not related to a general rhythmicity of thermal sensitivity.



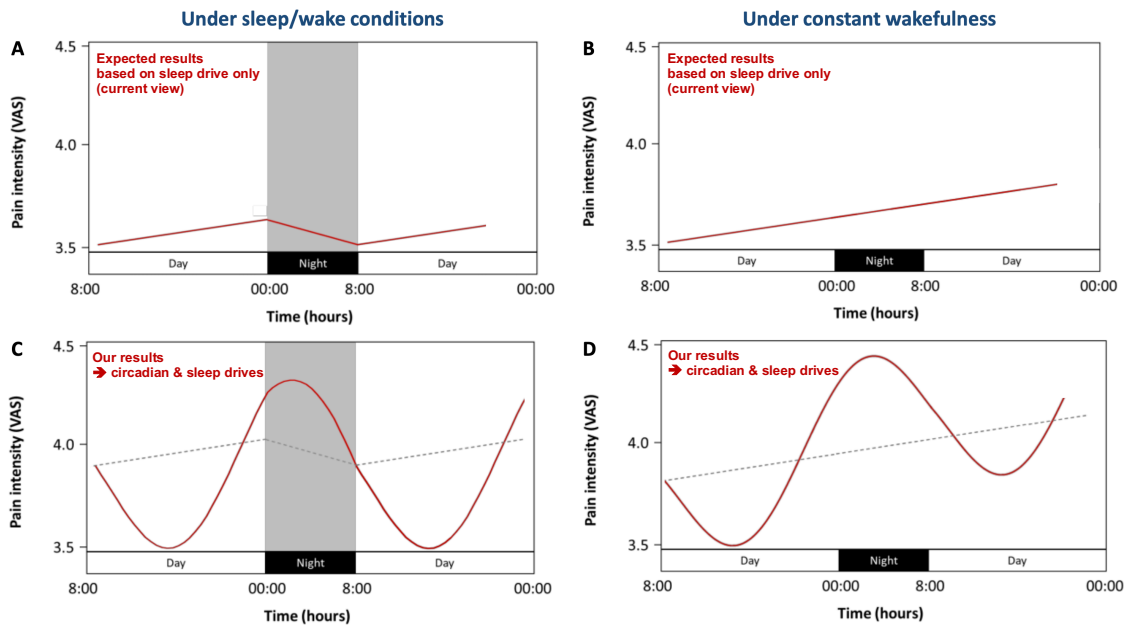
Supplementary Figure 2. Mean pain intensity in response to heat stimuli at 42 °C, 44 °C and 46 °C, across the 34-hour constant routine protocol (n = 12). Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO_n (mean DLMO_n ≈ 21:30). Combined models (sum of linear and sinusoidal components) of pain sensitivity in response to heat stimuli at 42 °C (light blue curve), 44 °C (blue curve) and 46 °C (dark blue curve). The amplitude changes (between peak and trough) in pain intensity for 42 °C, 44 °C and 46 °C are of 0.7, 0.8, and 1 VAS respectively.



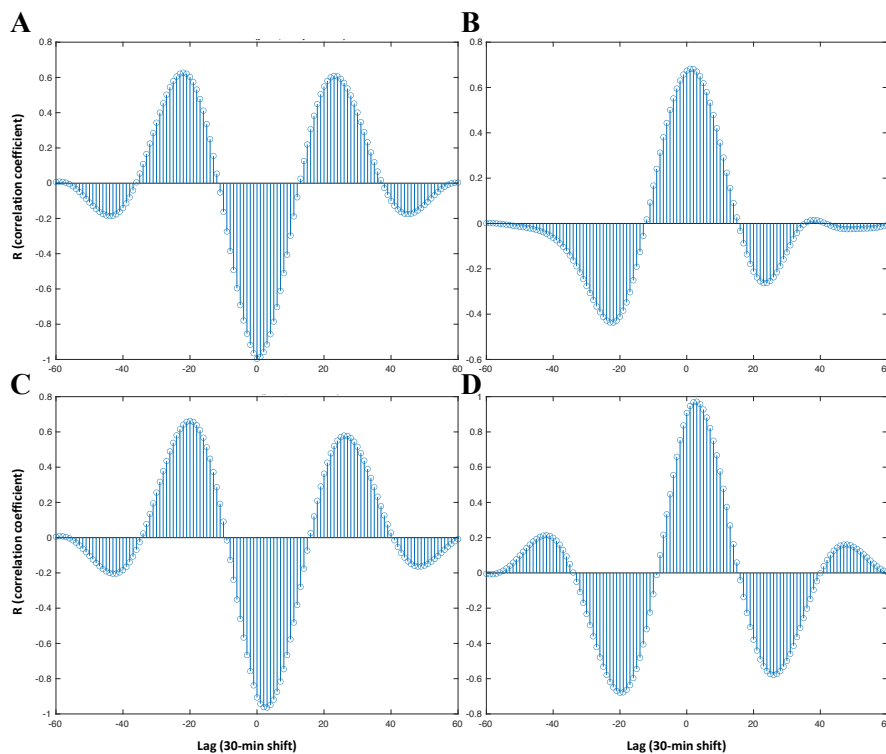
Supplementary Figure 3. Mean heat pain thresholds, across the 34-h constant routine protocol (n = 12). Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO_n (mean DLMO_n ≈ 21:30). Note that the Y axis is inverted scale so that higher pain is up and lower pain is down. **A.** Combined model (sum of linear and sinusoidal components) applied to normalized data (mean ± SEM; $R^2 = 0.69$). **B.** Linear component ($R^2 = 0.53$; $p < 0.01$). Pain sensitivity decreases with time spent awake. **C.** Sinusoidal component ($R^2 = 0.57$). Pain sensitivity follows a circadian rhythm with maximal pain at 4:30.



Supplementary Figure 4. Mean warm detection thresholds across the 34-h constant routine protocol (n = 12). Dark bars correspond to average habitual sleep episodes (biological night). Circadian time 0 corresponds to DLMO_n (mean DLMO_n ≈ 21:30). Note that the Y axis is inverted scale so that higher pain is up and lower pain is down. **A.** Combined model (sum of linear and sinusoidal components) applied to normalized data (mean ± SEM; $R^2 = 0.70$). **B.** Linear component ($R^2 = 0.68$; $p < 0.0001$). Heat sensitivity decreases with time spent awake. **C.** No sinusoidal component is found ($R^2 = 0.13$).



Supplementary Figure 5. Models of pain sensitivity across the 24h day. A-B: Expected pain sensitivity (red line) if pain was solely driven by the sleep/wake cycle (current homeostatic model based on data obtained from articles 3-5). Under regular sleep/wake conditions (A), pain sensitivity increases during wakefulness, in parallel to sleep pressure, and decreases during the night, in presence of sleep (gray area). Under constant enforced wakefulness conditions (B), pain sensitivity increases during wakefulness, keeps increasing at night in the absence of sleep, and continues increasing the next day. In this model, pain sensitivity only depends on time since awakening (linear increase during wakefulness), and time since bedtime (linear decrease during sleep). C-D: Our results show that pain sensitivity is driven by two independent and additive components: a sleep/homeostatic drive and a circadian drive. Under regular sleep/wake conditions (C) both mechanisms co-exist. Pain oscillates sinusoidally (strong circadian drive – red curve) over the 24 hours, and concomitantly increases linearly during wakefulness and decreases linearly during sleep (modest sleep/homeostatic drive – grey dotted line). Without sleep at night (D), our results under constant enforced wakefulness show the superimposed additive homeostatic (grey dotted line) and circadian drives of pain, with both a linear increase with time spent awake, and a sinusoidal oscillation.



Supplementary Figure 6. Cross-correlation analysis between the circadian components of pain sensitivity and temperature (A), melatonin (B), heart rate (C) and parasympathetic activity (RMSDD, D). All panels. Analysis computed on the sinusoidal components of each measure (as shown in Figure 4), and re-sampled at 30-min intervals (so that each lag equals a 30-minute delay or advance shift).

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