Duration invariance of the human circadian system response to pulsed light

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Abstract

The melanopsin-containing intrinsically photosensitive ipRGCs responsible for the synchronisation of the circadian clock with the environmental light-dark cycle are characterised by a delayed off-time following cessation of light exposure. In this work, we exploited this unusual physiologic property and interrogated how a sequence of flashes of bright light differing in duration could delay the human circadian clock in healthy young participants (n=27). Surprisingly, a sequence of 10 μs flashes (2,000 lux) delivered over the course of one hour leads to a phase shift equivalent to that observed after exposure to the same number of 10 s flashes at (2,000 lux), with no parametric relationship for intermediate flash durations. Our result demonstrates that the human circadian system can respond to light in a duration-invariant manner at very short durations, over six orders of magnitude, suggesting circadian responses to ultra-short flashes (sub-second) of light are saturated with even moderate photopic light levels.

Acknowledgements

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Introduction

The human circadian system is exquisitely sensitive to light. Light exposure in the evening and night can acutely suppress the production of melatonin [1-6], shift the phase of the circadian rhythm [5, 7-11], and modulate alertness and vigilance [12-14]. This effect is mediated by the retinal photoreceptors, with a major role played by a subset (<3%) of the retinal ganglion cells that express the short-wavelength-sensitive photopigment melanopsin, rendering them intrinsically photosensitive (ipRGCs = intrinsically photosensitive retinal ganglion cells) [15]. ipRGCs also receive cone and rod input [16], which contribute to a complex signal driving the circadian system.

The exact effect of a given light on the circadian system depends on its intensity, spectral distribution, and duration [17-19]. While experimental durations of light exposure are typically on the order of hours, it has been shown that sequences of 2-millisecond flashes of bright light (~1,700 lux) can induce phase shifts substantially larger than continuous light at the same illuminance [20]. Here, we examined to how short of a flash the human circadian system could respond by examining sequences of short-duration light flashes (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec; a 6 log unit range) and the consequent impacts on circadian phase, melatonin suppression, and alertness.

Results and Discussion

Using a pre-registered 16-day study design (two-week at-home, sleep and circadian stabilization protocol followed by constant posture protocols on sequential evenings [20]), we examined the effect of a regime of 240 moderately bright flashes (~2,000 lux) of broad spectrum white light of varying duration (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec; 15 second duration onset-to-onset) on the circadian timing of a group of healthy adult participants (n=28). Light was delivered to awake participants over 60 minutes starting at 4.07±1.16 hours after melatonin onset (after two hours of sleep). Circadian phase changes were calculated as the difference in salivary dim-light melatonin onset (DLMO), as determined by rise above baseline [21], during a constant posture protocol on Days 1 and 2 of the in-laboratory stay. The experimental stimulus was delivered using a spectrally and temporal calibrated retinal stimulation system delivering homogenous flashes over the entire visual field against a dark background (0 lux). In addition to change in DLMO, acute light-induced suppression of melatonin was calculated as the percent decline of melatonin concentrations between the beginning and end of the experimental stimulus. Acute changes in

subjective (Stanford Sleepiness Scale [22]) and objective (auditory version of the Psychomotor Vigilance Test [23]) alertness were also assessed immediately prior to and at the end of the light stimulus.

On average, we find phase delays in circadian timing on the order of 18±36 minutes ($t(26) = 2.5434$, $p = 0.0173$). Surprisingly, the effect is independent of the duration of flashes (Figure 1; estimated slope in simple linear intercept-slope model = 0.0025± 0.061, p=0.96684) over the entire range of durations spanning six orders of magnitude (1:1,000,000). Salivary melatonin was not suppressed by the light, but increased invariantly over all flash durations (average 11%±21% increase over the hour; $t(26) = 2.7242$, $p = 0.0114$; linear slope = -0.33±2.17, p=0.881). Both lapses on the auditory PVT (1.8±4.2 decrease over the hour, $t(26) = -2.2516$, p=0.033, linear slope = –0.31±0.42, p=0.4688) and subjective sleepiness measured by the SSS (average 0.74±1.6 point decrease; $t(26) = -2.4690$, $p = 0.0204$, linear slope -0.14±0.15, p=0.36632) exhibited duration-invariant improvements. Median reaction time on the auditory PVT did not change in response to the light flashes (23.1±66.7 ms decrease over the hour, $t(26) = -1.8003$, $p = 0.0834$, linear slope = -2.20±6.73, p=0.74667).

The ability of humans to consciously perceive light spans a very wide range of light intensities, from the sensitivity to single photons by the retinal rods [e.g. 24] to encoding fine spatial detail, colour and motion during daytime light levels. At detection threshold, the intensity and duration of a flash can be traded off against one another below a certain flash critical duration, leading to the same conscious perceptual performance if the product between the intensity and the duration is the same. For conscious perception, this temporal integration does not hold for flash durations over ~100 ms [25]. For shifting the circadian clock, however, it appears as though the mechanisms integrating light information are invariant to flash duration ranging from the microsecond to the multisecond time scale.

As was implied in early studies of circadian photoreception [26], in response to brief flashes of light, the circadian phase shifting system does not act as a photon counter. If the system did act as a photon counter, we would expect at least a monotonic increase in phase shift with increasing durations. Thus, the most parsimonious explanation for our results is that the full, or near full impact of a flash of 2000 lux of light on the circadian timing system is produced by the initial photon capture, in as short as 10 microseconds, if not less.

Our results are consistent with the hypothesis that 2000 lux of light is sufficient to saturate the response of the circadian system to a sequence of light flashes. Our bright short-duration stimuli seen against a dark background are biased to recruit the rods, which are known to

saturate at daylight levels [27-29] and provide synaptic input to the ipRGCs. However, our study cannot uniquely arbitrate between (early) rod-driven saturation at the photoreceptor level, or later neural saturation. Future work should tease apart the role of the different photoreceptors to circadian functions, at different time scales.

The changes in objective and subjective alertness are consistent with previous work [20] that reported an invariant increase in alertness in response to different interflash intervals, which had a dose-response relationship with circadian phase shifting. It is likely that the improvement in alertness observed in this protocol is a non-specific psychological response to the protocol (i.e., the flashes are aversive stimuli that increase wake, but a greater number or duration of flashes does not increase wake further).

As we continue to develop the technical abilities to craft light in terms of colour and intensity, the manipulation of temporal properties of light may become the new frontier to improve health and well-being. Our study shows how in principle, ultra-brief light flashes at the microsecond scale can be used to elicit phase shifts no different in magnitude than a regime of flashes delivering 100,000 times as many photons. Exploiting this surprising duration-invariance of human circadian photoreception could be used to address circadian desynchrony emerging in modern life, such as jet lag or shift work, in an energy-efficient way. Our results indicate that longer is not always better for the circadian system.

Materials and Methods

Pre-registration and deviations from pre-registered protocol. The study protocol was pre-registered on the Open Science Framework (https://osf.io/5sv53/) and registered at ClinicalTrials.gov (NCT01119365; "Bright Light as a Countermeasure for Circadian Desynchrony"). Notably, we deviated from the pre-registered study protocol by including an additional (10 sec) exposure duration. PSG data were collected but not analysed.

Ethical approval. The protocol was reviewed and approved by the Stanford University Institutional Review Board, conforming to the Declaration of Helsinki. Prior to any procedures, subjects signed informed consent forms.

Sample characteristics. 31 participants (13 female, 18 male) healthy, young (18-35 years) participants with normal weight with no somatic diseases, sleep disorders (Pittsburgh Sleep Quality Index, PSQI [30] $\leq$ 5), moderate chronotype (reduced Morningness-Eveningness Questionnaire, MEQ [31] $\geq$ 11 and $\leq$ 27), no history of substance abuse (Alcohol Use Disorders Identification Test, AUDIT [32] $\leq$ 7), no depressive symptoms (Center for

Epidemiologic Studies Depression scale, CES-D [33] ≤ 17), no use of hormonal contraceptives (females only), and normal colour vision (assessed with Ishihara Plates [34]) completed the study. We excluded two participants (1 female, 1 male) because of contaminated melatonin assays, one participant due to mistiming of light (female), and one participant because of accidental light exposure in the morning (female), yielding a total sample of 27 subjects (n=27, mean±1SD age: 25.7±3.94 years). The break-down of duration assignment was: 10 μs, 3 participants; 100 μs, 4 participants; 1 ms, 4 participants; 10 ms, 5 participants; 100 ms, 3 participants; 1 s, 4 participants; 10 s, 4 participants.

Design. Participants were exposed to a sequence of 240 light flashes of varying duration (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 s, 10 s) spaced 15 seconds apart (from onset to onset). Acute effects on melatonin suppression, objective alertness, subjective sleepiness, and electrophysiological correlates of arousal (polysomnography, PSG) were measured immediately before and at the end of the light exposure (LE). Effects of LE on circadian phase was measured as the change in melatonin onset determined on a constant posture protocol (CP1) prior light exposure and a constant posture protocol the following day (CP2).

Protocol. Participants took take part in a 16-day study protocol:

- Days 1-14: Participants were instructed to maintain a regular sleep and wake time schedule at home (±30 minute window of bed time and wake time). Sleep-wake patterns were monitored using an actigraphy device (Actiwatch2, Philips, Bend OR) and a self-reported sleep diary [35]. From these data, the midpoint of sleep (MSP) was estimated and used as the midpoint of the in-laboratory sleep opportunity.
- <u>Day 15</u>: The participant entered the laboratory during the late afternoon of Day 15. During the evening, the participant underwent the first constant posture procedure (CP1, 8 hours, beginning 8 hours before habitual bedtime). During this procedure, the participant was given isocaloric meals (Ensure, Abbott Laboratories, Chicago IL) every 60 minutes, yielding a total caloric intake matched to what they would have received during dinner (calculated using the Mifflin-St. Jeor formula; [36]). During CP1, objective alertness (auditory version of the Psychomotor Vigilance Task, PVT; [23, 37]) and subjective sleepiness (Stanford Sleepiness Scale, SSS) were measured every 60 minutes. Saliva was collected every 30 minutes in untreated polypropylene tubes. Four hours before MSP (typical bedtime), participants were given the opportunity to sleep in darkness. After 1 hour and 45 minutes of sleep time in darkness (2 hours and 15 minutes before MSP), participants were awakened in the dark. Saliva was collected and auditory

PVT and SSS were administered in the dark. Starting two hours before MSP, participants were exposed to a sequence of 240 full-field flashes for one hour (2000 lx; 10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 s, 10 sec) through a custom-made mask for light delivery. At 20, 40 and 60 minutes into the light exposure, saliva was collected. During the last 10 minutes of light exposure, the auditory PVT was administered, followed by an SSS. The mask was then removed from the participant and the participant continued to sleep in darkness.

- Day 16: The participant was awakened at their habitual wake time (4 hours after MSP) into a dimly light room (<10 lux) and received breakfast and lunch at usual times. During the evening, the participant had a second constant posture procedure (CP2, 10 hours in total, beginning 8 hours before habitual bedtime), during which the participant was given isocaloric meals every 60 minutes, yielding a total caloric intake matched to what they would have received during dinner (same number as on Day 15, but spread over 10 hours instead of 8). During CP2, objective alertness (auditory PVT) and subjective sleepiness (SSS) were measured every 60 minutes and saliva was collected every 30 minutes.

Light delivery. Binocular full-field flashes of differing durations (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 s, 10 s) were delivered to the observer. A custom-light mask was constructed using modified welding goggles (Jackson WS-80 Shade 5.0 Cutting Goggles; Kimberly-Clark Professional, Mississauga, ON, Canada) containing an acrylic panel with three horizontally arranged LED strips (12 SMD LEDs each, Lumileds L235-4080AHLCBAAC0). The light from the LEDs was diffused using a piece of diffusing acrylic (TAP Plastics, Mountain View, CA). For additional diffusion, the participant wore ping pong ball halves cut out to match the shape of the eye's orbit. The LEDs were pulsed using electronics developed in-house based on the Arduino Uno R3 microcontroller.

We verified the timing of our apparatus for the nominal flash durations 10 μs, 100 μs, 1 ms, 10 ms, 100 ms, and 1 s using an integrated photodiode and transimpedance amplifier (OPT101, Texas Instruments) connected to a digital oscilloscope (Tektronix TDS 2024C). We measured the logic-level control pulse sent from the microcontroller as well as the light output (Fig. S2). We averaged over 128 (10 μs), 128 (100 μs), 128 (1 ms), 64 (10 ms), 16 (100 ms), and 16 (1 s) pulses. The maximum amplitude of the pulse is approximately constant across all nominal pulse durations, indicating that there is no shift in light intensity due to duration. Integrating the light output, the logarithm of the integrated light output over the pulse duration is linear with the logarithm of the nominal pulse duration. Spectral output

was measured using a PR-670 spectroradiometer (Photo Research, Syracuse NY) (yielding calibrated radiance) for all stimulus durations, indicating stationarity of the spectrum across stimulus durations and across the entire stimulus protocol (240 flashes, 1h) (Fig. 1B, right panel).

Melatonin assay. Saliva (at least 1 mL) was collected using polypropylene tubes (Fisher Scientific, Hampton NH). Samples were centrifuged after collection, frozen at –20°C, then stored at –80°C within 24 hours. Salivary melatonin concentration was assayed according to the manufacturer's instructions using the Bu□hlmann direct saliva Melatonin ELISA Kits (ALPCO, Salem NH). For a given participant, all samples were assayed on the same plate.

Objective alertness: auditory PVT. We used a modified auditory psychomotor vigilance test (PVT; [23, 37]) to measure objective alertness using a serial collection of simple reaction times to auditory stimuli generated by a piezo buzzer. The stimuli were spaced apart in time at random inter-stimulus intervals (ISIs) between 2 and 6 seconds (discrete steps: 2, 3, 4, 5, 6 second ISIs). Upon button press, the tone stopped and the next trial began with a random ISI. Approximately 100 of these stimuli were presented, with the order of ISIs randomized at the beginning of the experiment. This assessment took 10 minutes. The auditory PVT was implemented using custom-made Arduino hardware and software. To measure response latencies, we modified sample code from a report validating using the Arduino platform to measure reaction times [38]. There is a 30-second time out which is considered a lapse trial. If there is a response during the ISI period, this was counted as an error of commission and the counter was reset, starting a new trial period. The random seed for the ISIs is initialized by reading analogue voltage noise from an unconnected pin in the Arduino.

Subjective alertness: SSS. Participants completed the Stanford Sleepiness Scale (SSS, [22]). The SSS is a single question assessment of current sleepiness that uses a 7-point Likert-like scale. Scale values range from 1 to 7, with higher values indicating greater subjective sleepiness.

Determination of phase shift. Phase shifts were determined by examining the acute change in the timing of salivary DLMO. This onset was determined by calculating the time at which the melatonin concentrations rose above a variable threshold (twice the average of the first three daytime samples). In cases in which this variable threshold was ambiguous (n=4), we used a curve-fitting method [39]. Phase shift was calculated as the DLMO on CP1 – DLMO on CP2, such that negative changes indicate a delay in timing.

Statistical analysis. We subjected our data to two sets of statistical analysis. For each measure, we estimated if there was a non-zero slope using a simple linear model with an intercept and slope term, with $\log_{10}$ flash duration as the predictor. A non-zero slope would indicate a monotonic relationship between flash duration and the measure. Additionally, we used single-sample *t*-tests on change scores to determine if there is any effect at all, irrespective of flash duration. The measures of interest were: phase shift (Fig. 2A), difference in melatonin pre- and post-LE (Fig. 2B), the difference in number of lapses in the auditory PVT pre- and post-LE (Fig. S1A), the difference in median reaction time pre- and post-LE (Fig. S2A), and the difference in sleepiness as assessed by SSS (Fig. S2B). Data are presented as mean ± SD.

Figures

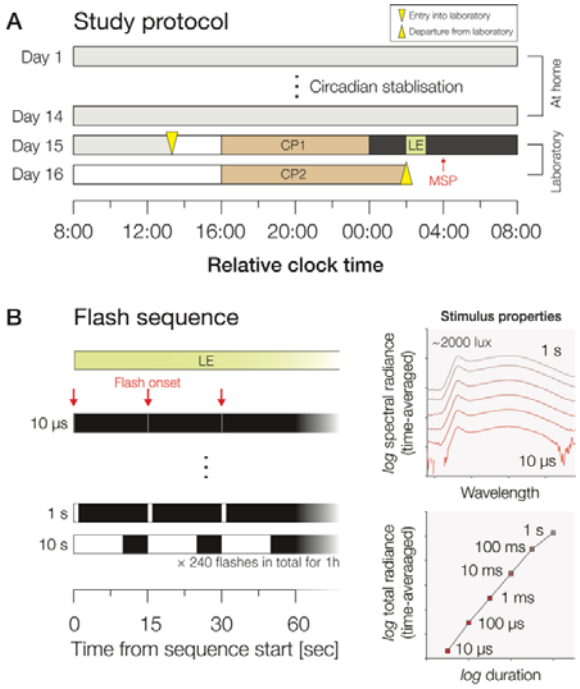

Figure 1. ***A*** Schematic diagram of 16-day study protocol. During CP1 and CP2, saliva samples were collected and behavioural assessments (objective alertness, subjective sleepiness) were performed. MSP = mid-sleep point; LE = light exposure; CP = constant posture. ***B*** Schematic diagram of flash sequence over the 1-hour light exposure phase. Flashes (~2,000 lux) were separated by 15 seconds onset-to-onset. Spectral properties of the stimulus were invariant of flash duration (*right column*, *top*), and total time-averaged radiance delivered was as expected (*right column*, *bottom*).

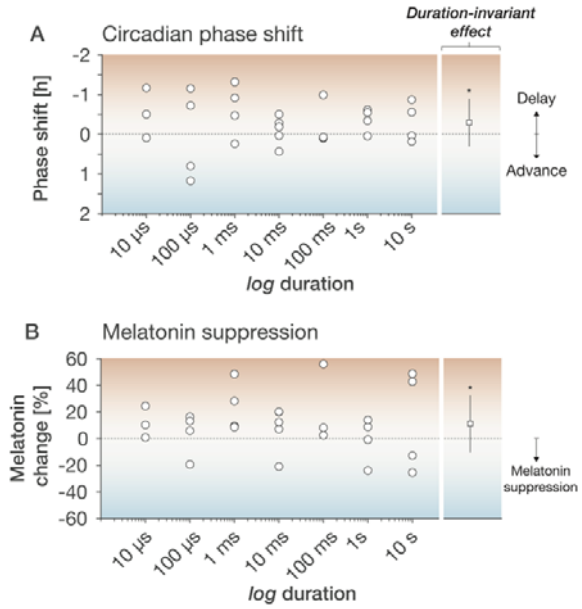

Figure 2. ***A*** Circadian phase shifted elicited by a 1-hour sequence of flashes different durations 10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec). Negative phase values indicate a delay, while positive phase shifts indicate an advance of the rhythm. ***B*** Acute melatonin suppression over the 1-hour light exposure. Asterisk indicates significance at the 0.05 level (one-sample t-test on change scores).

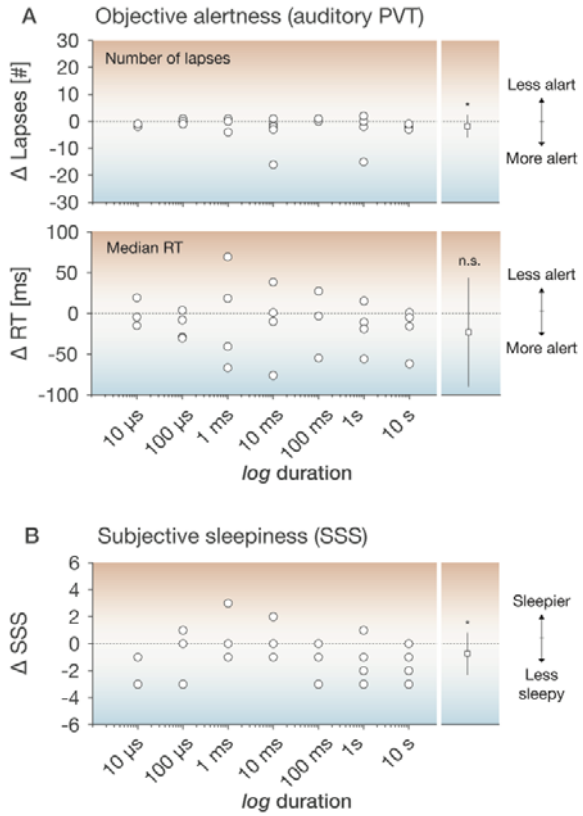

Figure S1. ***A*** Changes in objective alertness by a 1-hour sequence of flashes different durations 10 µs, 100 µs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec). *Top panel*: Number of lapses; *bottom panel*: median RT. ***B*** Changes in subjective sleepiness over the 1-hour light exposure. Asterisk indicates significance at the 0.05 level (one-sample t-test on change scores).

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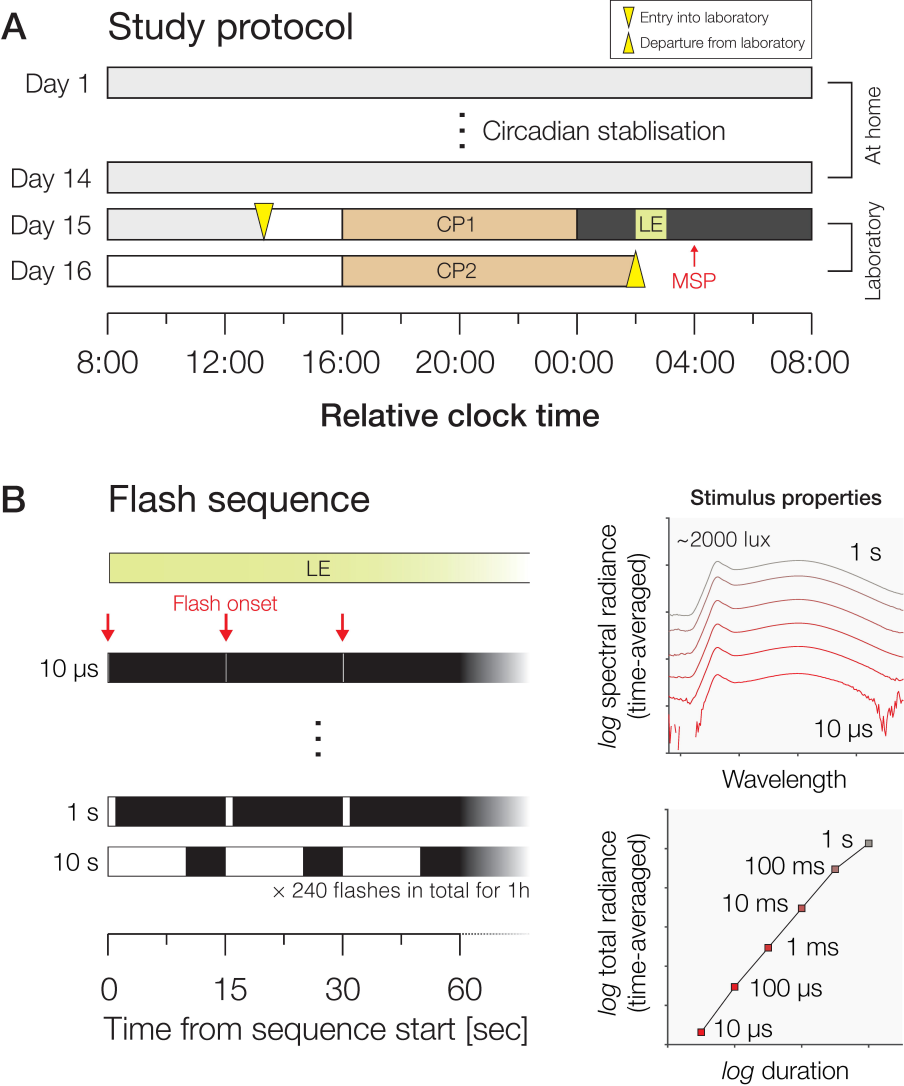
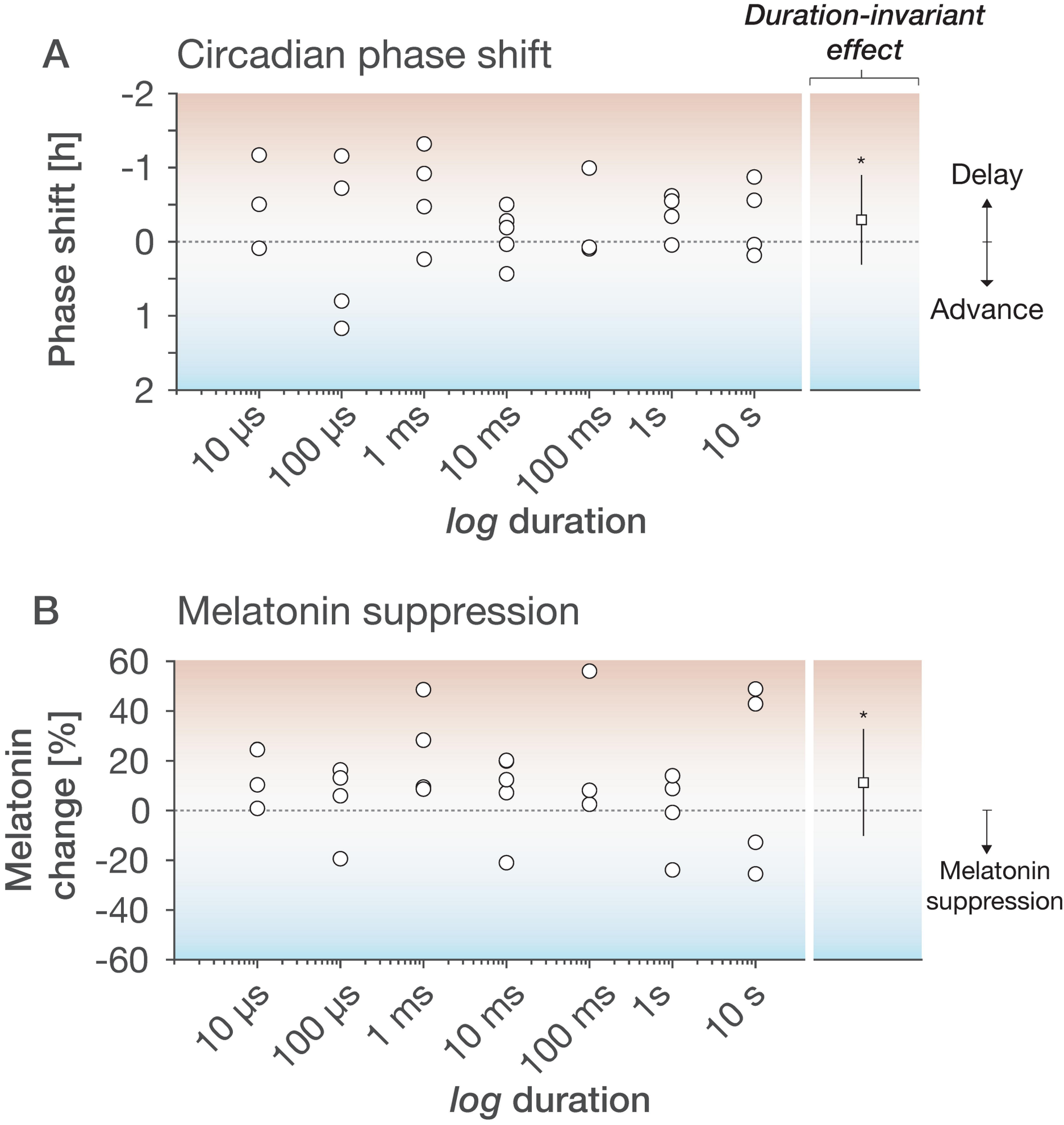
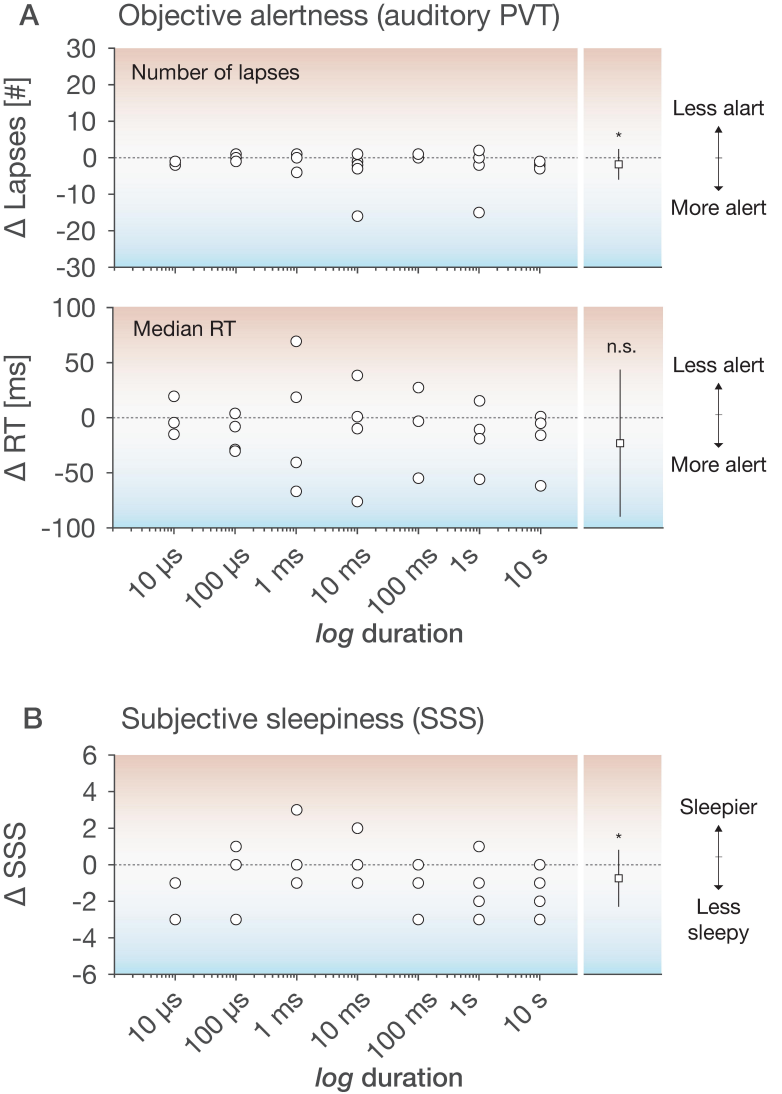

A
Study protocol
Entry into laboratory
Departure from laboratory
Day 1
Circadian stablisation
Day 14
At home
Day 15
CP1
LE
Day 16
CP2
MSP
Laboratory
8:00
12:00
16:00
20:00
00:00
04:00
08:00
Relative clock time
B
Flash sequence
LE
Flash onset
10 µs
1 s
10 s
× 240 flashes in total for 1h
0
15
30
60
Time from sequence start [sec]
Stimulus properties
~2000 lux
1 s
10 µs
log spectral radiance (time-averaged)
Wavelength
1 s
100 ms
10 ms
1 ms
100 µs
10 µs
log total radiance (time-averaged)
log duration


A
Circadian phase shift
Duration-invariant effect
Phase shift [h]
-2
-1
0
1
2
*
Delay
Advance
10 µs
100 µs
1 ms
10 ms
100 ms
1s
10 s
log duration
B
Melatonin suppression
Melatonin change [%]
60
40
20
0
-20
-40
-60
*
Melatonin suppression
10 µs
100 µs
1 ms
10 ms
100 ms
1s
10 s
log duration


A
Objective alertness (auditory PVT)
Number of lapses
Δ Lapses [#]
30
20
10
0
-10
-20
-30
*
Less alart
More alert
Median RT
Δ RT [ms]
100
50
0
-50
-100
n.s.
Less alert
More alert
10 µs
100 µs
1 ms
10 ms
100 ms
1s
10 s
log duration
B
Subjective sleepiness (SSS)
Δ SSS
6
4
2
0
-2
-4
-6
*
Sleepier
Less sleepy
10 µs
100 µs
1 ms
10 ms
100 ms
1s
10 s
log duration