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Significance Statement: Light:dark cycles (T-cycles) that are considerably different from Earth’s 24-h day are known to be challenging for our brain and body. But it is unclear if we can adapt to, and function normally under, T-cycles that are only slightly different (<1 h) from 24 h. By using laboratory mice as experimental models, we ask if the mammalian circadian system can adapt to the Martian photoperiod of 12.33-h light and 12.33-h dark (T24.66). Our study indicates circadian realignment is possible under T24.66 but comes at a cost: it changes sleep pattern, reduces brain signals associated with alertness, and affects short-term memory during the biological night. Our results highlight certain inevitable neurophysiological and functional changes among personnel participating in space exploration missions.

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Abstract

Aberrant light:dark cycles (T-cycles) that deviate considerably from our naturally evolved intrinsic period (τ) of ~24 h pose severe environmental challenges for the mammalian circadian system. But adaptability to T-cycles that deviate only slightly (<1 h) from T24, notably the Martian 12.33-h light:12.33-h dark cycle (T24.66) remains unexplored. By using laboratory mice, we examined the effects of T24.66 on circadian entrainment, ultradian rhythm, sleep and alertness, as well as hippocampus-mediated object memory. T24.66 lengthened τ , allowing rest–activity rhythm to realign with the slightly longer Martian photoperiod without free running. Circadian rhythmic power was not dampened under T24.66 but ultradian noise was amplified, as revealed by fast Fourier transform (FFT) and wavelet analysis. Despite circadian realignment, sleep pattern was altered with increased sleep at midnight due to an advance in the siesta peak. EEG spectral analysis revealed that waking EEG theta activity (8–12 Hz) was attenuated at night. These time-of-day dependent changes in sleep pattern and alertness were accompanied by attenuated short-term object memory at night, due to dysregulated response to familiar objects without affecting response to novelty. The T24.66 regime provides a promising approach to study the ramifications of (mal)adaptation to the Martian photoperiod for brain function.

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Light:dark cycles (T-cycles) that are considerably different from Earth's 24-h day are known to be challenging for our brain and body. But it is unclear if we can adapt to, and function normally under, T-cycles that are only slightly different (<1 h) from 24 h. By using laboratory mice as experimental models, we ask if the mammalian circadian system can adapt to the Martian photoperiod of 12.33-h light and 12.33-h dark (T24.66). Our study indicates that circadian realignment is possible under T24.66 but comes at a cost: it changes sleep pattern, reduces brain signals associated with alertness, and affects short-term memory during the biological night. Our results highlight certain inevitable neurophysiological and functional changes among personnel participating in space exploration missions.

1 Introduction

2 Life on Earth evolved under a rhythmic 24-h cycle of day and night (T24). As a result, organisms possess a light-
3 sensitive circadian pacemaker with an intrinsic period length (τ) that is close to, but not exactly, 24 h [1].
4 Laboratory studies in rodents have demonstrated that extreme T-cycles that deviate from τ , including T7, T20,
5 T22.5, T27, and T30, pose environmental challenges for the mammalian circadian system, affecting
6 photoentrainment [2, 3], sleep [4], cognitive function [2, 5], physical health [2, 3], and even longevity [6]. However,
7 adaptability to less extreme T-cycles that deviate only slightly (<1 h) from T24 and potential consequences of
8 (mal)adaptation remain largely unexplored. One prime example is the Martian photoperiod of T24.66 (12.33-h
9 light:12.33-h dark cycle that is ~40 min longer than T24), which is relevant to space exploration missions [7] and
10 has received increasing attention from astrobiologists, chronobiologists, and neuroscientists in recent years [8,
11 9]. Empirical findings from humans [7, 10], as well as formal predictions from the two-process model of sleep–
12 wake regulation [11], suggest that humans are capable of maintaining alignment with T-cycles that deviate slightly
13 (<1 h) from T24, reflecting the well-known (limited) plasticity in timing within the circadian range [1].

14 The adaptive advantage of such “circadian resonance” is that it allows internal processes to realign with T-cycles
15 that are close to our naturally evolved τ , thereby achieving functional optimization; this notion was first proposed
16 by C. S. Pittendrigh over 60 years ago [12, 13]. But even in certain situations where the T-cycle and τ are aligned
17 with each other, like in humans working under T24.66 in the Phoenix Mars Lander (PML) mission, brain functions
18 can still be compromised [7, 9]. Specifically, the field study by Barger *et al.* [7] suggests that certain cognitive
19 functions, such as sustained attention and visuospatial working memory [7], may be particularly vulnerable to
20 subtle deviations from T24; cognitive deficits can occur in cases where circadian misalignment is *not* evident.
21 This field study [7], however, lacked the necessary baseline control conditions to evaluate the extent of cognitive
22 deficits under T24.66. Thus, we aimed to verify these potential effects on alertness and memory in laboratory
23 mice, which are often used as experimental models for sleep and circadian rhythm disruption [14]. We posed
24 three related questions: (a) can the mammalian circadian system realign with T24.66 *without free running*, which
25 is typically observed under other extreme T-cycles [2, 3, 4]? (b) is there any repercussion for memory
26 performance despite normal circadian realignment under T24.66? (c) what is the electrophysiological signature
27 of such T24.66-induced memory deficit? To address these questions, we manipulated artificial light:dark cycles
28 to mimic the Martian T24.66 cycle in a controlled environment, employing continuous home cage activity
29 monitoring, non-invasive piezoelectric sleep recording, cortical electroencephalography, and memory
30 assessment to fully evaluate circadian rhythm and sleep pattern, as well as electrophysiological signatures of
31 attentional and memory deficits under T24.66.

32 Using wildtype C57BL/6 mice of both sexes, we reported the first investigation on the effects of T24.66 on
33 circadian entrainment, ultradian rhythm, sleep and electrophysiological markers of alertness, as well as
34 hippocampus-mediated short-term object memory requiring a functional central circadian clock [15]. We found
35 that although the mouse’s rest–activity rhythm could align with (i.e., did not free run from) T24.66, mice exhibited
36 changes in sleep pattern and waking EEG theta activity during their biological night. These changes in nocturnal

37 vigilance were accompanied by a decline in short-term object memory performance in both sexes, due to
38 dysregulated response to familiar objects without affecting response to novelty. Thus, the T24.66 paradigm
39 provides a promising approach to study the consequences of (mal)adaptation to the Martian photoperiod, with
40 the potential to reveal sexually dimorphic (mal)adaptive circadian and cognitive responses.

41 Results

42 **T24.66 lengthens activity period and triggers circadian realignment**

43 To examine the effects of T24.66 on circadian period—a behavioral readout of the central circadian clock [1]—
44 we applied fast Fourier transform (FFT) to the home cage locomotor activity record under each T-cycle and
45 defined the intrinsic period length (τ) within the circadian range as the dominant period between 20 h and 30 h
46 in the FFT spectrum. As anticipated, T24.66 caused period lengthening in both sexes [main effect of T-cycle in
47 ♀: $F(1,5) = 26.563, p = 0.004$; main effect of T-cycle in ♂: $F(1,5) = 501.018, p < 0.001$]. This effect resembles
48 lengthening of melatonin metabolite τ in humans working under the PML mission, as activity τ from mice under
49 T24.66 (range in ♀: 24.603–24.769 h; range in ♂: 24.675–24.925 h) fell within the range of melatonin metabolite
50 τ from humans in the PML mission (range: 23.93–24.83 h) [7, their Table 1]. Thus, our lighting regime can
51 simulate the basic effect of Martian photoperiod on τ lengthening in experimental animals.

52 Given that τ can “resonate” with T24.66 in mice—reflecting some plasticity in timing within the circadian range
53 [1]—we then examined if the phase of daily activity was realigned with the new light:dark cycle and the stability
54 of such circadian realignment. Daily activity midpoints were used as a phase marker to examine phase shift [16];
55 example PIR activity actograms with extracted midpoints from a mouse are displayed in **Figure 1A**. Under
56 T24.66, activity midpoints (**Figure 1B**) were reflected as gradual phase delays relative to the T24 photoperiod in
57 both sexes [T-cycle × Day interaction in ♀: $F(1,5) = 24.342, p = 0.004$; T-cycle × Day interaction in ♂: $F(1,5) =$
58 15.916, $p = 0.010$; T-cycle × Day × Sex interaction: $F(1,10) = 1.979, p = 0.190$]; a similar effect was found on
59 sleep–wake cycles in a separate cohort of mice (**Figure 2A**). However, when T24.66 data were plotted on the
60 local Mars clock (**Figure 1C**), phase delays were no longer evident [T-cycle × Day interaction in ♀: $F(1,5) =$
61 4.426, $p = 0.089$; T-cycle × Day interaction in ♂: $F(1,5) = 0.323, p = 0.595$; T-cycle × Day × Sex interaction:
62 $F(1,10) = 2.289, p = 0.161$]. This shows that the mouse’s rest–activity rhythm can realign with T24.66 without
63 free running, in line with the data from humans in the PML mission [7].

64 **T24.66 amplifies ultradian noise without dampening circadian power**

65 Although Barger *et al.* [7] examined various circadian metrics, they did not assess ultradian (>24 h) rhythms;
66 these “noises” from peripheral oscillators could be unmasked as a result of circadian realignment [16, 17]. To
67 investigate this possibility, a T-cycle × Harmonics (12 h, 8 h, and 6 h) ANOVA was conducted on normalized FFT
68 power of ultradian harmonics at 12 h, 8 h, and 6 h (data pooled across sexes). This analysis revealed a main
69 effect of T-cycle [$F(1,11) = 9.684, p = 0.010$] but no T-cycle × Harmonics interaction [Greenhouse–Geisser
70 corrected $F_{\text{G}}(2,22) = 2.567, p = 0.126$], indicating that ultradian rhythmic power was generally amplified under
71 T24.66. Although there was an increase in ultradian noise under T24.66, circadian power was *not* dampened

72 ([Figure 1D](#) versus [Figure 1E](#)). FFT spectra of group-average PIR activity are shown in [Figures 1F](#) and [1G](#),
73 where FFT power is normalized to the 24-h peak, visualizing the increase in power at the 8-h harmonic under
74 T24.66 ([Figure 1G, top](#)); wavelet analysis [18] revealed a similar effect ([Figure 1G, bottom](#)).

75 **T24.66 increases sleep at midnight due to an advance in the siesta peak**

76 Changes in ultradian rhythm could be related to alterations in sleep patterns. To examine the effect of T24.66 on
77 sleep, in four out of seven male mice (*cohort 2*) sleep was assessed non-invasively *via* floor piezoelectric sensors,
78 which allow us to infer sleep states by detecting 1–4 Hz rhythmic signals generated from respiration-induced
79 chest movement in an asleep mouse [19]; in the remaining three male mice, sleep was assessed *via* cortical
80 EEG. For both experiments, light and dark phases were split into twelve 60-min bins under T24 or 61.66-min
81 bins under T24.66; sleep amount was quantified in each hourly bin. During the light phase, there was no effect
82 of T-cycle on piezoelectric sensor-defined sleep [main effect of T-cycle: $F(1,11) = 0.113, p = 0.759$; T-cycle ×
83 Hourly Bin interaction: $F(11,33) = 0.945, p = 0.512$] ([Figure 2B](#)). By contrast, in the dark phase there was a T-
84 cycle × Hourly Bin interaction [$F(11,33) = 8.105, p < 0.001$], due to increased sleep at midnight as a result of a
85 slight advance in the siesta peak under T24.66 (bin 6: $p < 0.001$; bin 7: $p = 0.047$) ([Figure 2E](#)). Increase sleep
86 at midnight was corroborated in a separate cohort of tethered EEG-implanted mice [bin 7 for both NREM and
87 REM sleep: $p < 0.001$ and $p = 0.022$, respectively; T-cycle × Hourly Bin quadratic trend in within-subjects contrast
88 test: $F(1,2) = 33.621, p = 0.028$] ([Supplementary Table 1](#)).

89 **T24.66 attenuates waking EEG fast theta power in a time-of-day dependent manner**

90 Barger *et al.* [7] reported that sustained attention (as assessed by the psychomotor vigilance task) was
91 compromised under T24.66 and subjective ratings of fatigue were worsened in a time-of-day dependent manner.
92 To examine the electrophysiological signature of these effects, we analyzed frontal electroencephalography
93 (EEG) activity in the waking EEG spectrum across the slower theta (5–8 Hz) and faster theta range (8–12 Hz).
94 EEG spectral analysis showed that increased nocturnal sleep was accompanied by reduced frontal waking EEG
95 power in the faster theta range (8–12 Hz) during the biological night, but not in the light phase [T-cycle × Diurnal
96 Phase × Frequency Bin interaction: $F(20,40) = 2.043, p = 0.027$] ([Figures 2C,2D](#) versus [Figures 2F,2G](#)). This
97 effect was more pronounced in the second half of the dark phase [main effects of T-cycle in 1st half and 2nd half
98 of the dark phase: $F(1,2) = 1.201, p = 0.387$ and $F(1,2) = 25.436, p = 0.037$, respectively; T-cycle × Frequency
99 Bin interactions in 1st half and 2nd half of the dark phase: $F(20,40) = 0.518, p = 0.942$ and $F(20,40) = 11.337, p <$
100 0.001 , respectively] ([Figures 2F](#) versus [2G](#)). By contrast, there was no effect of T-cycle in the slower theta range
101 (5–8 Hz) at night [main effect of T-cycle: $p = 0.346$; T-cycle × Frequency Bin interaction: $p = 0.202$]. As waking
102 EEG fast theta activity is associated with locomotion (e.g., spatial navigation) [20] and attentiveness (e.g., “online”
103 processing of salient cues) [21], our data indicates reduced nocturnal alertness under T24.66.

104 **T24.66 attenuates short-term object memory due to dysregulated response to familiarity**

105 To investigate the cognitive consequence of reduced nocturnal alertness, a naïve cohort of 12 mice (*cohort 3*)
106 received the spontaneous object recognition task, a hippocampus-mediated memory task [22] that is sensitive

to lighting manipulation [5, 16, 17], sleep disturbance [23], and circadian rhythm disruption (such as in circadian arrhythmic hamsters and $Cry1^{-/-}Cry2^{-/-}$ mice) [15, 24]. Object trials were given repeatedly on multiple nights (1 trial per night; 4 trials in total) under each T-cycle. Under the baseline T24 condition, mice discriminated between familiar *versus* novel objects [$F(1,11) = 25.471, p < 0.001$] and object memory performance was delay dependent (5 min *versus* 2 h), as indicated by the Object Type \times Sample–test Delay interaction [$F(1,11) = 16.090, p = 0.002$]. Under T24.66, mice continued to show object discrimination [$F(1,11) = 13.918, p = 0.003$], but performance was no longer delay dependent [Object Type \times Sample–test Delay interaction: $F(1,11) = 0.004, p = 0.950$]. A T-cycle \times Minute of Test ANOVA conducted on the object discrimination index [$t_{\text{novel}}/(t_{\text{familiar}} + t_{\text{novel}})$] revealed a main effect of T-cycle in the first two minutes of the test phase [mean discrimination indices under T24 *versus* T24.66: 0.683 ± 0.025 *versus* 0.592 ± 0.031 ; $F(1,11) = 7.112, p = 0.022$], confirming that short-term object memory was attenuated under T24.66 (Figure 2H). Further analyses showed that familiar object exploration was increased by $\sim 40\%$ at test under T24.66 [T24 *versus* T24.66: $7.640 \pm 0.841 \text{ s min}^{-1}$ *versus* $10.554 \pm 1.145 \text{ s min}^{-1}$; $F(1,11) = 15.479, p = 0.002$] (Figure 2I), whereas novel object exploration was unaffected [T24 *versus* T24.66: $16.598 \pm 1.272 \text{ s min}^{-1}$ *versus* $14.937 \pm 0.906 \text{ s min}^{-1}$; $F(1,11) = 1.019, p = 0.334$] (Figure 2J). This indicates that T24.66 leads to dysregulated response to familiar objects without affecting response to novelty.

While in previous studies mice that were circadian arrhythmic ($Cry1^{-/-}Cry2^{-/-}$) showed impaired memory due to a general loss of interest in investigating objects [24], the effect of T24.66 in our study was *not* due to the same reason, as object exploration rates in the sample phase were elevated under T24.66 [T24 *versus* T24.66: $10.004 \pm 0.392 \text{ s min}^{-1}$ *versus* $10.918 \pm 0.452 \text{ s min}^{-1}$; $F(1,11) = 5.272, p = 0.042$]. The same level of exploration was expressed toward the familiar object at test under T24.66, indicating that mice did not habituate to the familiar object across sample to test phases [$10.918 \pm 0.452 \text{ s min}^{-1}$ *versus* $10.554 \pm 1.145 \text{ s min}^{-1}$; $F(1,11) = 0.189, p = 0.672$]. This is in contrast to the familiar-object habituation effect across sample and test phases under T24 [$10.004 \pm 0.392 \text{ s min}^{-1}$ *versus* $7.640 \pm 0.841 \text{ s min}^{-1}$; $F(1,11) = 15.850, p = 0.002$], leading to a T-cycle \times Phase interaction [$F(1,11) = 9.067, p = 0.012$]. This confirms that the decline in object memory is due to a lack of habituation to familiar objects without altering the mouse's interest toward novel objects.

132 **T24.66 gives rise to sexually dimorphic circadian responses but comparable cognitive responses in both 133 sexes**

In addition to T-cycle effects, there were some sexually dimorphic circadian responses to T24.66 in C57BL/6 mice. Under T24, activity τ was shorter in females than in males [$\text{♀}: 23.606 \pm 0.054 \text{ h}; \text{♂}: 24.201 \pm 0.084 \text{ h}; t(10) = 5.964, p < 0.001$], but T24.66 lengthened τ by ~ 30 min and >1 h in male and female mice, respectively [$\text{♀}: 24.816 \pm 0.031 \text{ h}; \text{♂}: 24.690 \pm 0.028 \text{ h}; t(10) = 2.981, p = 0.014$]. We also found that female but not male mice responded to T24.66 with an increase in circadian rhythm, as indicated by T-cycle \times Sex interactions for circadian power [$F(1,10) = 42.768, p < 0.001$] as well as τ [$F(1,10) = 7.573, p = 0.020$] (Figure 1D). Furthermore, the T-cycle \times Harmonics \times Sex ANOVA conducted on normalized ultradian power at 12 h, 8 h, and 6 h found a T-cycle \times Sex interaction [$F(1,10) = 6.416, p = 0.030$], due to a stronger T-cycle effect on ultradian power amplification in male than female mice (Figure 1E). As our study was designed to investigate the effects of T24.66 (which

were quantitatively but *not* qualitatively different between sexes) rather than to reveal sexually dimorphic circadian responses, we did not pursue this further. Like previous memory studies with C57BL/6 mice [25, 26] we also found a sex difference in object memory at night [mean discrimination indices in ♀ *versus* ♂: 0.594 ± 0.031 *versus* 0.681 ± 0.021 ; $F(1,10) = 5.399$, $p = 0.043$]. This did not interact with T-cycle [main effect of T-cycle: $F(1,10) = 6.924$, $p = 0.025$; T-cycle \times Sex interaction: $F(1,10) = 0.710$, $p = 0.419$], suggesting that the T-cycle effect on object memory was comparable between sexes (**Figure 2H**).

149 Discussion

150 **T24.66 causes period lengthening and circadian realignment but amplifies ultradian noise**

151 Like humans [7, 10], female and male mice were capable of adjusting their activity period and maintaining a
152 stable phase under the Martian photoperiod. The stability of entrainment under T24.66 is in contrast to free-
153 running activity and body temperature rhythms of mice under more extreme T-cycles, such as T20 and T30 [2,
154 3, 4]; this is consistent with the notion of (limited) plasticity in circadian timing [1]. Although T24.66 did not dampen
155 the power (amplitude) of circadian rhythm, it amplified ultradian noise at 12-h and 8-h harmonics. The underlying
156 mechanism of these ultradian behavioral rhythms is likely to be multifaceted [27, 28, 29], but they could be driven
157 partly by endogenous peripheral oscillators—such as hepatocytes that possess molecular clocks with intrinsic
158 periods close to 12 h and 8 h (e.g., genes involved in metabolic processes) [28, 29]—due to changes in feeding-
159 related behavioral patterns.

160 **T24.66 reveals potential sexual dimorphism in circadian responses: Current versus previous mouse 161 studies**

162 Like some previous studies using the same mouse strain (C57BL/6) at similar ages (2.5–4 months old), we found
163 differences in daily activity rhythms between female and male mice. In one previous study [30], female mice
164 showed shorter wheel-running activity τ under free-running conditions (constant darkness); this is consistent with
165 the shorter locomotor activity τ in our female mice under T24. Although both female and male mice showed τ
166 lengthening under T24.66, the τ lengthening effect was more pronounced in female mice. In addition, our female
167 mice showed increased circadian power under T24.66, whereas male mice responded to T24.66 with an increase
168 in ultradian but not circadian power. Our result is thus in line with the stronger circadian activity rhythm in female
169 mice in previous studies under T24 [31, 32] as well as under a long-term misaligned lighting schedule (weekly
170 8-h phase advance) [32]. This latter study also showed that female mice were less susceptible to the detrimental
171 effects of weekly 8-h phase advance, as indicated by their stronger circadian rhythms in daily activity and hepatic
172 transcriptome [32]. Therefore, there could be sexually dimorphic responses under certain lighting conditions;
173 although both female and male mice showed stable entrainment (as indicated by activity midpoints) under T24.66.

174 **T24.66 has repercussions for alertness and short-term memory despite circadian realignment: 175 Laboratory mice versus humans**

176 Despite the discrepancy in methods assessing alertness and cognitive function in human and animal studies, a
177 cross-species comparison may shed light into the common cognitive consequences of T24.66. In male mice,

178 T24.66 increased sleep at night (but not during the light phase) and reduced waking cortical EEG fast theta
179 activity—which is associated with navigation [20] and “online” processing of salient cues in the awake state [21].
180 The reduction in alertness at night under T24.66 was accompanied by reduced habituation to familiar objects
181 and a decline in short-term object memory in both female and male mice. Our results are partly consistent with
182 data from humans participating in the PML mission, in which personnel synchronized their work schedule to
183 T24.66 [7]. PML participants reported increased sleepiness and fatigue, accompanied by reduced attention in
184 the psychomotor vigilance task (PVT) and serial reaction time task (SRT) and a decline in short-term visuospatial
185 memory in the matching-to-sample task (M2S). These cognitive effects in PML participants were dependent on
186 preceding sleep history (i.e., stronger effects with extended wakefulness) and the circadian phase at which
187 assessments were conducted (i.e., stronger effects at night) [7]. Similarly, mice under T24.66 showed attenuated
188 frontal EEG fast theta activity in the awake state—an effect that was also dependent on time of day as well as
189 diurnal phase. Our results thus corroborate the findings from Barger *et al.* [7], providing some preliminary insights
190 into the electrophysiological underpinnings of attentional and short-term memory deficits in humans under
191 T24.66 that can be verified in future studies. Taken together, our T24.66 paradigm in laboratory mice provides a
192 promising comparative approach to study (mal)adaptation of the mammalian circadian system to the Martian
193 photoperiod, as well as offering the potential to reveal sexually dimorphic (mal)adaptive circadian and cognitive
194 responses.

195 **Short-term object memory changes under T24.66 could be regulated by sleep history and glutamate 196 AMPA receptors: A unified framework based on previous mouse studies**

197 A recently proposed framework has provided some clues on potential mechanisms underlying the effects of
198 T24.66 on object habituation and short-term memory [33]. Habituation is one of the most important forms of non-
199 associative learning [34, 35]; it occurs when an animal receives repeated presentation of the same stimulus,
200 often manifested as a reduction in behavioral response to the familiar stimulus. In the object recognition task,
201 short-term habituation leads to reduced attention to the familiar object, thereby allowing attentional processing
202 of the novel object. This behavioral response is known to be dependent on glutamate receptors, as deletion of
203 the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1 subunit in *Gria1*^{-/-} mice
204 impairs short-term object memory [34, 35]. Critically, glutamate AMPA receptor GluA1 subunit levels are
205 regulated by preceding sleep history [36, 37, 38]—accumulating sleep debt leads to overexpression of
206 postsynaptic GluA1 subunits and may cause dysregulated attentional processing, which can be renormalized by
207 recovery sleep [33]. Thus, sleep-dependent changes in GluA1 levels could be one of the potential mechanisms
208 underlying reduced object habituation in T24.66; this remains to be tested in future studies.

209 **Methods**

210 **Animals, housing conditions, and study design**

211 A total of 32 C57BL/6 mice (12♀ and 20♂) were used in this study. They were at least 10 weeks old at the start
212 of the experiment. Mice were housed individually in enclosed wooden chambers equipped with cool white LED
213 lights (50–250 lux), providing artificial 12-h light:12-h dark cycles (T24 baseline) and subsequently 7–18 days of

12.33-h light:12.33-h dark cycles mimicking the Martian photoperiod (T24.66). Daily locomotor activity (*cohort 1*), sleep pattern and waking EEG (*cohort 2*), and object memory (*cohort 3*) were assessed using this within-subjects design. Throughout the study mice had *ad libitum* access to food and water, and the ambient temperature in the animal holding room was maintained at $22 \pm 1.5^{\circ}\text{C}$. Experimental procedures were approved by the Duke Kunshan University Institutional Animal Care and Use Committee and conducted at the Duke Kunshan University—The First People's Hospital of Kunshan Joint Brain Sciences Laboratory in accordance with the standard of the Association for Assessment and Accreditation of Laboratory Animal Care International.

Cohort 1: Locomotor activity monitoring

In the first cohort of mice (6♀ and 6♂ C57BL/6J), locomotor activity data were collected continuously in 10-s bins with the passive infrared sensor (PIR) system designed by Brown *et al.* [39]. From the PIR record, circadian period and phase of entrainment were determined under each T-cycle. The circadian period length of locomotor activity was defined as the dominant period between 20 h and 30 h in the FFT spectrum (R package `spectr`) [40]. Circadian amplitude between 23.5–24.5 h and ultradian harmonic amplitude at 11.5–12.5 h, 7.5–8.5 h, and 5.5–6.5 h were also quantified from FFT spectra. Activity midpoints were defined as time points at which PIR activity in the preceding 8 h and activity in the subsequent 8 h were equivalent. For each bin (*i*) the difference (Δ_i) between preceding 8-h activity and subsequent 8-h activity was determined, and the product of Δ_i and Δ_{i-1} was calculated. The bins in which $\Delta_i\Delta_{i-1} < 0$ and $\Delta_{i-1} < 0$ were defined as midpoints [16].

Cohort 2: Sleep monitoring

In the second cohort of mice, sleep was assessed by piezoelectric film sensors (4♂ C57BL/6J) and by cortical EEG (4♂ C57BL/6N). For four mice, piezoelectric film sensors (Signal Solutions, Kentucky) were placed on the cage floor, and sleep was monitored continuously for at least 7 days under each T-cycle. Sleep–wake decision statistics were determined every 2 s in SleepStats (Signal Solutions, Kentucky) based on the characteristics of the piezo signal. Rhythmic low-amplitude piezo signals within the 1–4 Hz breathing rate of an asleep mouse favored decisions of sleep, whereas irregular high-amplitude piezo signals due to locomotor activity favored decisions of wakefulness [19].

For the remaining four mice, an EEG/electromyography (EMG) head mount (KD-EEG/EMG-Ag, Kedou Brain Computer Technology, Jiangsu, China) was implanted under isoflurane anesthesia; the implanted head mount had silver wires for EEG and stainless steel wires for EMG. Stainless steel screw electrodes were implanted epidurally over the frontal cortex (AP +1.0 mm and ML –1.0 mm), parietal cortex (AP +2.5 mm and ML –2.5 mm), and cerebellum; the latter served as the reference electrode. Silver wires from the head mount were connected to the screw electrodes, which provided two EEG derivations (frontal *versus* cerebellum and parietal *versus* cerebellum). Stainless steel wires from the head mount were inserted into the neck muscle for EMG. Screw electrodes and head mount wires were secured on the skull with bone cement. After recovery from surgery, one EEG-implanted mice died during the recording phase of the experiment due to a water bottle leak in its home cage; as such, only 3 male mice completed the EEG experiment.

249 After at least 10 days of recovery, EEG-implanted mice were acclimatized to the experimenter's handling and
250 tethering procedure prior to the start of the experiment. EEG and EMG data were collected with a sampling rate
251 of 2 kHz using the CerePlex Direct acquisition system (Blackrock Neurotech, Utah). Mice were first kept under
252 T24 for a week, and EEG and EMG data were recorded for 24 h on the last day. After the end of the first recording
253 period, mice were kept under T24.66 in the second week, during which EEG and EMG were recorded for 24.66
254 h on the last day. Acquired EEG and EMG data were filtered between 0.1–15 Hz. Filtered signals from the frontal
255 EEG derivation were used for sleep scoring and EEG spectral analysis. EEG power spectra were computed
256 using FFT. Classification of vigilance states (wake, NREM, and REM) was based on bandpass-filtered frontal
257 EEG and EMG data for each 4-s epoch and was conducted in AccuSleep, a convolutional neural network (CNN)-
258 based pipeline developed and validated by Barger *et al.* [41].

259 **Cohort 3: Object memory testing**

260 The third cohort of mice (6♀ and 6♂ C57BL/6J) received the spontaneous object recognition memory task. Under
261 T24 and after a week of T24.66, behavioral testing was conducted at night (2–4 h after the dark phase onset) in
262 a 30 cm × 30 cm × 30 cm arena that was illuminated by cool white LED light of <5 lux. The arena was decorated
263 with distinct visual patterns as in our previous studies, to facilitate the use of visual cues to guide object choice
264 [16, 17, 42]. Mice were first given 10-min acclimatization trials in an empty arena for two nights; then they
265 received object trials for four nights (one trial per night). Two of the object trials had a 5-min sample–test delay
266 and the remaining two trials had a 2-h delay. On each trial, the mouse explored two identical replicates of an
267 object for 10 min in the sample phase. After a 5-min or 2-h delay, it was returned to the arena and was given 3
268 min to explore another replicate of the previously encountered object *versus* a novel object in the test phase.
269 After four trials of object testing under T24, mice were kept under T24.66 for at least seven days; another four
270 object trials were then given at night (one trial per night) under T24.66. Real-time tracking of object exploration
271 was conducted in ANY-maze (Stoelting, Illinois), which tracked the mouse's head position every 100 ms. Total
272 object exploration times were recorded in sample and test phases.

273 **Statistical analysis**

274 Mean values ± standard errors of the mean were reported. Parametric analyses with $\alpha = 0.05$ were conducted
275 in SPSS (IBM) and R. Primary analyses were within-subjects and split-plot analyses of variance (ANOVAs).
276 Within-subjects factors in ANOVAs included T-cycle, time (days, hours, or minutes), and frequency (or the
277 reciprocal of frequency), with Sex as the between-subjects factor. For effects involving within-subjects factors
278 with more than two levels, Greenhouse–Geisser corrections were applied to the degrees of freedom (df)
279 whenever the assumption of sphericity was violated; F values with adjusted df were denoted as F_{ϵ} . Significant
280 interaction terms from ANOVAs were followed up with (uncorrected) simple effect analyses in SPSS [43].

Data Availability Statements

All data from this study will be shared in response to reasonable requests to the corresponding authors (S.K.E.T. and S.C.K.). Data underlying Figures 1 and 2 and R codes used to generate these figures will be available in a repository (Figshare) and accessible via a DOI link prior to publication of the manuscript.

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Supplementary Table 1: EEG-defined sleep (in percentages) in the first half of the dark phase

(a) NREM sleep

Mouse ID	T-cycle	Hourly Bin						
		1	2	3	4	5	6	7
EEG-m002	T24	5.33	3.22	0	41.00	18.00	0.22	40.56
	T24.66	0	46.70	29.19	46.16	60.50	53.41	60.43
EEG-m003	T24	2.44	0	0	33.89	28.00	64.11	54.67
	T24.66	57.58	52.54	73.30	60.76	76.52	40.00	73.19
EEG-m004	T24	47.56	46.11	50.56	40.67	22.56	40.00	38.89
	T24.66	0	1.84	20.22	49.83	16.02	36.86	53.08
Mean	T24	18.44	16.44	16.85	38.52	22.96	34.78	44.70
	T24.66	19.19	33.69	40.90	52.25	51.01	43.42	62.23**

**Within-subjects contrast $p < 0.01$

(b) REM sleep

Mouse ID	T-cycle	Hourly Bin						
		1	2	3	4	5	6	7
EEG-m002	T24	0	0.22	0	7.00	0.44	0	1.44
	T24.66	0	5.62	0.54	3.68	7.03	1.95	5.41
EEG-m003	T24	0	0	0	5.00	2.33	7.33	9.33
	T24.66	0	2.92	2.16	4.97	9.20	1.19	13.41
EEG-m004	T24	6.33	5.67	9.11	6.44	2.67	6.56	6.11
	T24.66	0	0	1.95	6.16	0.11	7.14	8.54
Mean	T24	2.11	1.96	3.04	6.15	1.81	4.63	5.63
	T24.66	0	2.85	1.55	4.94	5.45	3.42	9.12*

*Within-subjects contrast $p < 0.05$

Figure Legends

Figure 1. Circadian rhythm is entrained to T24.66 but ultradian noise is amplified at the 8-h harmonic.

A: Double-plotted actograms of home cage PIR activity from a male mouse; daily activity midpoints are indicated by white dots. **B** and **C:** Activity midpoints (6♀ and 6♂) under T24.66 plotted on the T24 clock (*Uncorrected*; panel **B**) versus local Mars clock (*Corrected*; panel **C**); dashed diagonal lines in panel **B** demarcate onset and termination of the dark phase under T24.66. **D:** Circadian period length and FFT power within 23.5–24.5 h; dashed horizontal lines indicate reference lines of 24 h and 24.66 h. **E:** Normalized FFT power at 12-h, 8-h, and 6-h harmonics. Star symbols in panels **D** and **E** indicate significant effects of Sex and T-cycle from ANOVAs ($p < 0.05$). **F** and **G:** FFT (**top**) and wavelet spectra (**bottom**) of group-average PIR activity record in male mice; power values in FFT and wavelet spectra are normalized to the peak power value in the corresponding spectrum. In **top** panels, the dashed vertical line indicates the reference line of 24 h and inverted triangles indicate ultradian harmonics at 12 h, 8 h, and 6 h in the FFT spectrum. In **bottom** panels, dashed vertical lines indicate periods at 24 h, 12 h, and 8 h in the wavelet spectrum. Ultradian 8-h harmonic power was elevated in both FFT and wavelet spectra under T24.66 (panel **G**, **top** and **bottom**).

Figure 2. Increased nocturnal sleep due to an earlier siesta is accompanied by attenuated waking EEG fast theta activity and a decline in hippocampus-mediated short-term object memory performance at night. **A:** Non-invasive assessment of sleep–wake cycles using piezoelectric sensors (4♂); data from T24.66 are plotted on the uncorrected timescale to visualize the change in sleep–wake cycles relative to T24. **B:** Piezoelectric sensor-defined sleep amount in hourly bins in the light phase; T24.66 data are plotted on the local Mars clock (i.e., each hourly bin lasts for 61.66 min). **C** and **D:** Normalized waking frontal EEG fast theta activity (8–12 Hz) in the first and second halves of the light phase (3♂); EEG power is normalized to total power in the waking EEG spectrum. **E:** Piezoelectric sensor-defined sleep in the dark phase (same mice in panel **B**). **F** and **G:** Normalized waking EEG fast theta activity in the first and second halves of the dark phase (same mice in panels **C** and **D**). **H:** Object discrimination performance at night (6♀ and 6♂); the effect of Sex did not interact with the effect of T-cycle on object memory. **I:** Fold changes in familiar object exploration under T24.66 relative to T24. **J:** Fold changes in novel object exploration under T24.66 relative to T24. Star symbols indicate significant effects of T-cycle from within-subjects ANOVAs ($p < 0.05$).



