Physiological and cognitive consequences of a daily 26 h photoperiod in a primate: exploring the underlying mechanisms of the circadian resonance theory

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The biological clock expresses circadian rhythms, whose endogenous period (tau) is close to 24 h. Daily resetting of the circadian clock to the 24 h natural photoperiod might induce marginal costs that would accumulate over time and forward affect fitness. It was proposed as the circadian resonance theory. For the first time, we aimed to evaluate these physiological and cognitive costs that would partially explain the mechanisms of the circadian resonance hypothesis. We evaluated the potential costs of imposing a 26 h photoperiodic regimen compared to the classical 24 h entrainment measuring several physiological and cognitive parameters (body temperature, energetic expenditure, oxidative stress, cognitive performances) in males of a non-human primate (*Microcebus murinus*), a nocturnal species whose endogenous period is about 23.5 h. We found significant higher resting body temperature and energy expenditure and lower cognitive performances when the photoperiodic cycle length was 26 h. Together these results suggest that a great deviation of external cycles from tau leads to daily greater energetic expenditure, and lower cognitive capacities. To our knowledge, this study is the first to highlight potential mechanisms of circadian resonance theory.

1. Introduction

Circadian rhythms provide notable benefits to the organism compared with passive oscillations in local and transient response to exogenous factors [1,2]. The ubiquity of biological clocks in living organisms suggests a high adaptive value enhanced by the synchronization of behavioural and physiological processes with optimal phases of the cyclic environment [1] and by the coordination of internal rhythms with each other [3,4]. The master clock controls vital metabolic cycles [5] and synchronizes every day with the environmental zeitgeber (from german zeit: time and geben: giver), the most important being the light-dark cycles, whose period $T$ is 24 h. Light information is transmitted through retinal photoreceptors to the suprachiasmatic nuclei, where the central clock is based, which synchronizes the whole organism via chemical pathway, such as hormones [6–8].

Without any light clue, the circadian clock expresses its own periodicity, close to 24 h, called the free-running period or tau [9,10]. Tau is both individual- and species-dependent [11], and maintained intra-cellularly by transcriptional and translational feedback loops regulating the expression of the clock genes [12,13]. First proposed by Pittendrigh & Minis [14], the circadian resonance theory assumes a relationship between tau and fitness. Longevity would be enhanced when the free-running period is close to the period of environmental cycles, when tau and $T$ ‘resonate’: the more tau gets close to 24 h, the longer the longevity. Indeed, drosophila reared under photoperiodic regimens far from 24 h displayed reduced lifespan [14]. These historical experiments were the first to confirm a negative link between the deviation of tau from 24 h and longevity. Even though it seems very intuitive, the circadian resonance adaptive advantage is little shown...
in mammals. Wyse et al. [15] found a negative correlation between the deviation of tau from 24 h and longevity in several strains of laboratory mice, and several species of rodents and primates; Libert et al. [16] showed that mice with circadian period close to 24 h lived about 20% longer than those with shorter or longer tau. These two studies provide evidence that keeping a 24 h free-running period positively affects lifespan. However, the underlying mechanisms that would explain the disadvantages of a desynchrony between tau and external light–dark cycles remain totally unknown. An assumption advanced is that daily marginal metabolic or physiological costs, required by the clock daily entrainment, would accumulate over life and with time, impact negatively longevity. Our study aimed at determining if these daily costs could be detected and quantified.

To address this issue, we focused on a non-human primate, the grey mouse lemur (Microcebus murinus). This small Malagasy lemur is an emerging model in neurosciences since it displays aged-related impairments similar to those found in humans (e.g. spontaneous neurodegenerative diseases or cognitive deficiencies [17]), including circadian rhythms alteration, such as locomotor activity (LA) fragmentation or sleep deterioration (see [18] for review). On the other hand, its small body size and light body mass make it an ideal and promising laboratory model. Regarding circadian features, the grey mouse lemur is strictly nocturnal and its metabolism is highly dependent on photoperiod [19]. Its mean free-running period lies around 23.5 h [20].

We submitted mouse lemurs to two different photoperiodic regimens, corresponding to a standard deviation of tau (individuals kept in light–dark cycles of 24 h) and a great deviation of tau (individuals kept in light–dark cycles of 26 h). We considered that clock daily entrainment may exert a direct or indirect influence on physiological and metabolic functions, particularly on basal metabolic activity, which might affect longevity. We thus focused on several factors that reflect this basal metabolism (e.g. oxygen respiration, energy expenditure, body temperature, oxidative stress) and measured them before and after photoperiodic treatments. Knowing that the circadian clock substantially influences cognitive performance [21], we were also wondering if daily synchronization costs could affect cerebral abilities. We hence assessed cognitive performance using a learning task based on visual discrimination.

2. Results

(a) Effects of photoperiodic treatments on locomotor activity and body temperature

T24 and T26 animals were entrained to the light–dark cycles they were submitted to, since their actograms were in phase with external zeitgeber (LA mean periods of 23.99 ± 0.01 for the T24, \( t_{T24} = -2.53, p_{T24} = 0.35 \), and 25.99 ± 0.04 for the T26, \( t_{T26} = -0.75, p_{T26} = 0.47 \); figure 1). Periodograms peaked at 24 h and 26 h in T24 and T26, respectively (figure 1). The
diagrams of temperature (see electronic supplementary materials, S1) present the same patterns (mean periods of 24.01 ± 0.01 and 25.98 ± 0.07, respectively; \( t_{T24} = 1.83, p_{T24} = 0.15; t_{T26} = -0.80, p_{T26} = 0.44 \)). Moreover, the phase angle of T24 and T26 individuals was not different before or after treatment, even though time significantly reduced the phase angle (time: \( F = 5.16, p = 0.04 \), treatment: \( F = 0.02, p = 0.88 \), time × treatment: \( F = 0.06, p = 0.81 \)).

No significant difference was observed in activity levels between T24 and T26 animals before or after treatment, but time had a positive effect on both groups during the active phase (table 1 and figure 2). Regarding body temperature (Tb), interactions between time and treatment were not significant, neither in resting nor in active phase. However, when subtracting the thermic fall, i.e. considering only Tb values of the resting phase after the daily minimal Tb \((T_{min})\) was reached, T26 animals exhibited significantly higher Tb than T24 after treatment (table 1 and figure 2).

### (b) Effects of photoperiodic treatments on calorimetric parameters

Even if all individuals exhibited a mass increase, both groups did not exhibit any significantly different mass gain (table 2). When considering the active phase, VO\(_2\), VCO\(_2\) and heat levels were not significantly different between treatments, but VCO\(_2\) significantly decreased with time in both groups (table 2 and figure 3). During the resting phase, the two-way ANOVAs showed a tendency for higher VO\(_2\) and VCO\(_2\) and heat in T26 individuals, regardless of time (table 2). Moreover, significant interactions between time and treatment revealed a significantly higher VO\(_2\) and VCO\(_2\) and a tendency to higher heat in T26 compared with T24 after treatment (table 2 and figure 3).

### (c) Effects of photoperiodic treatments on oxidative stress

Two-way ANOVAs did not reveal significant differences between groups in plasma 8OHdG levels (time: \( F = 0.24, p = 0.62 \), treatment: \( F = 0.59, p = 0.44 \), time × treatment: \( F = 0.06, p = 0.80 \); electronic supplementary material, figure S4).

### (d) Effects of photoperiodic treatments on learning performances

Among the 22 animals, 7 reached the success criterion: 3 in T24 and 4 in T26. The T26 individuals needed significantly more trials to reach the criterion than the T24 (17.5 ± 1.5 versus 7 ± 3.33, \( p = 0.03 \); figure 4).

### (e) Correlations between physiological, metabolic and cognitive parameters after treatment

In addition to the fact that most of calorimetric parameters were strongly correlated, oxidative stress was also positively correlated and tended to be correlated with the resting VO\(_2\) and VCO\(_2\), respectively. Furthermore, the number of trials needed to reach the success criterion in the cognitive task was positively correlated to resting VO\(_2\) and VCO\(_2\) (i.e. the higher resting VO\(_2\) and VCO\(_2\), the worse the cognitive capacities were). Finally, body temperature after thermic fall was positively correlated with resting VCO\(_2\) and tended to correlate positively with resting VO\(_2\) and negatively with cognitive performances (electronic supplementary material, table S2).

### 3. Discussion

In the present study, we aimed at investigating the metabolic costs of the circadian clock daily synchronization and its cognitive consequences. Our results suggest that an energetic and cognitive cost is daily generated when the free-running period \( \tau \) is much shorter than the environment periodicity. Animals submitted to light–dark cycles of 26 h, inducing a great divergence between \( \tau \) and external cycles, exhibited higher resting body temperature, higher VO\(_2\), VCO\(_2\) and heat levels and lower cognitive performances. This higher energetic and cognitive expenditure in T26 could be due to a higher requirement to keep the clock reset. To our knowledge, this is the first time that a study provides some insights on the potential biological costs of clock daily synchronization. The latter had been first evoked by Pittendrigh & Minis [14], then by Wyse et al. [15], in order to elucidate the circadian resonance mechanisms, but had never been assessed so far.
Animals in T24 and T26 were entrained to their light–dark treatments and random feeding times over the day prevented the animals from being synchronized by food distribution. Nevertheless, we verified that T26 animals were entrained to their photoperiodic regimen without masking effect. Although LA was restricted to daily dark period, it cannot be ruled out that other internal body rhythms may be desynchronized with external environment. Tb rhythm of T26 individuals

Figure 2. (a–c) Mean locomotor activity and body temperature during the resting phase, and mean body temperature during the resting phase after the thermic fall (i.e. \(T_b > T_{min}\)) in T24 and T26 before and after treatment. (d,e) Mean locomotor activity and body temperature during the active phase in T24 and T26 before and after treatment. (f,g) Daily profiles of locomotor activity and body temperature in T24 and T26 after treatment. Switch between night and day is represented by the black line. In order to overlap T24 and T26 graphs, \(x\)–axis of T26 animals was scaled to fit the T24 animals’ one. Each tick of each \(x\)–axis represents 2 h. arb. units = arbitrary units. For the daily profiles of LA and Tb before treatment, see electronic supplementary material, figure S2.

Table 2. Statistical results of two-way ANOVAs with repeated measurements (time × treatment) for body mass, \(VO_2\), \(VCO_2\) and heat during resting and active phases.

| parameters          | time                  | p       | treatment       | time × treatment | p       |
|---------------------|-----------------------|---------|-----------------|------------------|---------|
| body mass (g)       | 10.10                 | 0.001** | 0.13            | 0.75             | 0.01    | 0.91   |
| \(VO_2\) (ml.kg\(^{-1}\).h\(^{-1}\)) | 0.19                  | 0.25    | 2.65            | 0.10             | 3.75    | 0.05** |
| \(VCO_2\) (ml.kg\(^{-1}\).h\(^{-1}\)) | 0.27                  | 0.70    | 3.47            | 0.07             | 13.21   | 0.001**|
| heat (kcal.h\(^{-1}\)) | 1.54                  | 0.23    | 3.75            | 0.07             | 2.97    | 0.08   |
| \(VO_2\) (ml.kg\(^{-1}\).h\(^{-1}\)) | 0.79                  | 0.36    | 0.10            | 0.74             | 0.37    | 0.54   |
| \(VCO_2\) (ml.kg\(^{-1}\).h\(^{-1}\)) | 5.38                  | 0.02*   | 0.01            | 0.94             | 0.25    | 0.62   |
| heat (kcal.h\(^{-1}\)) | 0.08                  | 0.73    | 0.10            | 0.73             | 1.10    | 0.30   |
displayed a period of 26 h, and T24 and T26 animals did not exhibited a significantly different phase angle. In other words, T26 animals were entrained to their light–dark cycles and did not free-run. Despite its non-independent link with LA, body temperature can inform on organism entrainment. In most experiments where $T$ was modified and individuals’ rhythms decoupled from external cycles, body temperature and LA were not synchronized, LA being restricted to activity periods and body temperature free-running [22–26], which was not the case in our data. Moreover, experiments on entrainment limits in mouse lemurs conducted in our laboratory show that LA and temperature start decoupling over 27 h (M. Perret, personal communication, 2019), suggesting that 26 h T-cycles lie within the mouse lemur range of entrainment.

We observed that T26 animals displayed significantly higher body temperature and higher energy expenditure (VO$_2$, VCO$_2$ and heat) during the resting phase, especially after the daily metabolic fall. Higher body temperature was neither the consequence of a higher LA during the same period. Nor is it due to a potential adaptation time to 26 h cycles because the effect was found regardless of the considered week, even after three weeks of photoperiodic treatment (data not shown). A lot of studies on modified T-cycles were carried out on various species but only a few of them dealt with daily metabolic and physiological consequences of the organism’s entrainment to T-cycles different from 24 h, and none focused on metabolic rate or body temperature. In invertebrates, two species of Camponotus ants exhibited significantly faster pre-adult development under T24 compared to T20 and T28, suggesting a fitness advantage of ‘resonating’ clock [27]. Short-period mutant hamsters (whose ‘natural’ endogenous period lies around 24.05 h [28]) displaying a 22 h free-running period died younger and exhibited severe cardiac and renal diseases when raised under cycles of 24 h [29]. Vilaplana et al. [30] showed that rats kept under

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**Figure 3.** (a–c) Mean VO$_2$, VCO$_2$ and heat during the resting phase in T24 and T26 before and after treatment. (d–f) Mean VO$_2$, VCO$_2$ and heat during the active phase in T24 and T26 before and after treatment. (g–i) Daily profiles of VO$_2$, VCO$_2$ and heat in T24 and T26 after treatment. Switch between night and day is represented by the black line. In order to overlap T24 and T26 graphs, x-axis of T26 animals was scaled to fit the T24 animals’ one. Each tick of each x-axis represents 2 h. For the daily profiles of VO$_2$, VCO$_2$ and heat before treatment, see electronic supplementary material, figure S3.

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**Figure 4.** Trials needed before reaching the success criterion to the learning task. T26 individuals needed significantly more trials than T24 individuals to reach the criterion. Asterisk: $p < 0.05$. 
T25 and T26 light–dark cycles during 64 days exhibited lower body weight, less food intake and less efficiency (i.e. relationship between body weight increase and food intake) than rats kept under T24 cycles. This last experiment could draw an interesting parallel with our results, since assessed parameters are comparable to those we measured. Nevertheless, the theory behind these observations is actually totally opposite to our own hypothesis. Indeed, Vilaplana and co-workers started from the postulate that cycles of 25 or 26 h are closer to the rat’s free-running period than 24 h and that rats better ‘resonate’ under this cycle length. In this regard, higher available energy is preferentially allocated to activity and metabolism, rather than to growth efficiency. On the contrary, we suggest that individuals kept close to resonance frequency would consume less energy to maintain their clock reset and display a lower metabolism, which contradicts Vilaplana’s hypothesis. Furthermore, the hypothesis of a ‘better resonating clock under T25 and T26 cycles’ can be questioned, since the free-running period of rats is approximately 24.5 h [31].

Among endotherms it has been widely accepted that body temperature is directly influenced by metabolic rate, as this is the principal origin of endogenous heat [32,33]. In this respect, our calorimetric results are in line with body temperature observations. Indeed, VO₂, VCO₂, heat and Tb were significantly higher in T26 than in T24 animals during the resting time that closest resembles the basal metabolic state of the animal. Can a higher metabolic rate be regarded as a metabolic ‘cost’? According to the rate of living theory, the longevity of an organism is conditioned by its rate of metabolism [34]. Indeed, intraspecific negative relationships between longevity, ageing and basal metabolic rate have been found out in numerous species [35,36]. Therefore, the higher metabolic rate of T26 treatment could represent a fitness disadvantage and thus, a metabolic cost. As for body temperature, a lot of studies confirm that lower body temperature leads to slower ageing and longer lifespan in poikilotherms as well as in homeotherms [37]. For example, Hert-UCF2 mutant mice, displaying a reduction of Tb of 0.5–0.6°C demonstrated up to a 20% increase of median lifespan [35]. In humans, the Baltimore Longitudinal Study of Aging (BLSA) reported a lower body temperature related to higher lifespan and other positive physiological effects [38]. Here, we can also assume that the higher body temperature exhibited by the T26 animals represents a metabolic cost that would affect fitness in the long term.

The rate of living theory has then been further extended into the free-radical theory of ageing stating that ageing results from accumulating damages produced by reactive oxygen species, such as 8OHdG [39]. Our results did not exhibit any significant divergent plasma 8OHdG levels between the different photoperiodic groups. The light–dark treatments provided to the animals may have not been compelling or long enough to impact significantly the individuals at the DNA level. It highlights that daily costs imposed by the circadian clock resetting remain marginal at the cellular level. Further investigations could then consist in extending the duration of the metabolic stress imposed by the light–dark cycles. Indeed, we cannot totally rule out that the impairments on metabolic parameters would have been greater after more than three weeks of treatment.

Regarding cognitive outcomes, only seven animals reached the success criterion to the learning task, which is one-third of the total number of individuals. This result may seem low but it lies close to the success rate observed in other experiments using the same cognitive apparatus [40,41]. The resulting interpretations must though be viewed very cautiously. Among animals that learned the task, T26 animals needed significantly more trials to reach the success criterion than the T24, which can be seen as a cognitive cost due to a higher mismatch between the endogenous period and the environment. A higher required energy to reset the circadian clock may potentially create a side-effect in cognitive performances, highlighting a probable trade-off between metabolism and cerebral abilities. It is well documented that a strong link exists between circadian clock and cerebral performances. Through its link with the hippocampus [42,43], the circadian system influences mood, learning, time-place association and memory in laboratory mice [23,44,45], and the involvement of clock genes is well established [46]. Furthermore, numerous data indicate that circadian disorganization (jet-lag, phase shifts, ageing alterations, sleep impairments, shift work, etc.) invariably leads to impaired cognitive performances which suggests that clock resynchronization indirectly impacts cognitive capacities [47–49]. Only a very few studies have tested the effects of a chronic misalignment between tau and T, without sleep–wake cycles decoupled from circadian rhythm. Neto et al. [50] reported decreased performances in a passive avoidance memory task in rats kept under 22 h light–dark cycles compared to control group (L : D 24 h). The authors of this study raised the involvement of the circadian clock in an emotional component of the memory task, related to fear or risk evaluation. The cognitive cost observed in T26 animals could also suggest a lack of sleep, or at least sleep modification. LA profiles are not significantly different between T26 and T24 groups even though there seems to be a period between 24 and 26 h during which T26 animals activate (figure 2f). This non resting period could be associated with a slight sleep debt, which, cumulated over 20 days, could alter learning performances. A prolonged sleep deprivation affects cognitive performances, as it has been shown in humans [51,52], even if the sleep deprivation is moderate [53,54]. In this perspective, a cumulative sleep debt due to sooner activation of T26 animals could explain the worse results during the discrimination task, without being directly related to a daily synchronization cost. The altered cognitive performances of T26 animals may also be due to an improper entrainment to the imposed light–dark cycles. Although LA and body temperature were synchronized with each other, other internal rhythms might be desynchronized, suggesting a masking effect of light on LA and correlated body temperature, as previously mentioned. A lot of studies report the negative effect of body rhythms desynchrony on neuro-behavioural functions, especially during ageing, when the biological clock undergoes severe alterations [48,55]. In that case, weaker learning performances in T26 animals would rather be due to a potential internal desynchrony rather than to a cost of synchronization.

Although the inter-correlations between cognitive, cellular and metabolic parameters suggest a multi-scale effect of photoperiodic treatments on tested individuals, the precise mechanisms between clock daily synchronization and metabolic costs remain so far unknown and can only be hypothesized. They besides require the cellular mechanisms of light entrainment, that remain barely investigated [56]. In laboratory conditions (i.e. under constant intensity of light), clock entrainment is supposed to follow a discrete model, where light activation and extinction are supposed to mimic dawn and
Figure 5. Experimental design. Animals’ metabolic activity (calorimetry) was first assessed before treatments during 5 days, period during which pre-treatment blood was also collected. The animals were then separated into two different photoperiodic regimens: in light–dark cycles of 24 h (L : D = 14 : 10) or in light–dark cycles of 26 h (L : D = 15 : 11). After 15 days, metabolic activity was measured again and new blood sampling was collected. The animals then performed a learning task during 2 days.

4. Material and methods

(a) Animals

All cages were equipped with wood branches for climbing activities as well as wooden sleeping boxes. The ambient temperature and the humidity of the rooms were maintained at 25–27°C and at 55–65%, respectively. When they are not involved in experimental protocols, animals in facilities are exposed to an artificial photoperiodic regimen consisting of alternating periods of six months of summer-like long days (light–dark (L : D) 14 : 10) and six months of winter-like short days (L : D 10 : 14), in order to ensure seasonal biological rhythms [62]. The following experiment focused on animals during the long-day season.

(b) Experimental procedure

Twenty-two male grey mouse lemurs between 2 and 4 years-old were chosen randomly in the colony. Before the start of the treatment (figure 5), the animals were isolated under light–dark cycles of 24 h (L : D 14 : 10); the metabolic activity was measured using a calorimetry system during 5 days, followed by blood sampling to assess individual oxidative stress. We then submitted the animals to two different photoperiodic treatments during 22 days. Individuals of the first group (T24, n = 11) were kept under light–dark cycles T of 24 h with 14 h of light (resting phase) and 10 h of darkness (active phase) per day (L : D 14 : 10). Mouse lemurs of the second group (T26, n = 11) were submitted to light–dark cycles T of 26 h (L : D 15 : 11), leading to a great divergence between their free-running period and the environmental periodicity. After 15 days, metabolic activity was measured again during 5 days in the indirect calorimetry set-up, followed by blood sampling. Afterwards, the animals performed a cognitive task during the last 2 days of experiment. Animals were fed at random times of the day with fresh fruits and a homemade mixture, corresponding to an energy intake of 25.32 kcal day$^{-1}$ (see Dal-pan et al. [63] for details). Food intake and body mass were daily monitored, and Tb and LA were recorded continuously using telemetry implants during the whole experiment.
(c) Indirect calorimetry system
Metabolic activity was recorded using an indirect calorimetry system (Oxymax, Columbus Instruments, Columbus, Ohio, USA). Animals were housed in individual metabolic cages during 5 days (with 1 day for acclimation before measurements). Oxygen consumption (VO₂), carbon dioxide production (VCO₂) and energy expenditure (heat = 3.815 × VO₂ + 1.232 × VCO₂; see Lusk [64]) were recorded continuously. VO₂ and VCO₂ were expressed as a function of the whole body mass (ml h⁻¹ kg⁻¹) and heat in kcal h⁻¹.

(d) Body temperature and locomotor activity telemetric monitoring
Recording of LA and body temperature was obtained by telemetry. A small telemetric transmitter weighing 2.5 g (model TA10TA-F10, DataScience Co. Ltd, Minnesota, USA) was implanted into the visceral cavity under isoflurane anaesthesia (4% for induction and 1–1.5% for maintenance). After surgery, animals returned to their home cage and were allowed to recover for 15 days before the start of experiment and continuous recordings of LA and Tb. Temperature was punctually monitored every 5 min and LA was continuously recorded by a receiver placed into the cage, which detected vertical and horizontal movements and transmitted data to the computer (coordinate system, DataquestLab Prov. 3.0, DataScience). LA data were summed in intervals of 5 min and expressed in arbitrary unit (arb. units). Activity onset was defined as the first six successive bins where activity was greater than the mean LA during the resting phase. Periods of activity rhythms were calculated over the 20 first days of photoperiodic treatment (the last two days were excluded to avoid the influence of the cognitive task on LA and Tb data), using the Lomb-Scargle periodogram (LSP) procedure [65], with Clocklab software (Actimetrics, Evanston, IL, USA). LA, Tb and phase angle values were averaged over the 5 days before treatment, and over the 20 first days of photoperiodic treatment (the last 2 days were excluded as well). The phase angle was assessed calculating the period of time between lights on and minimal body temperature (Hmin). In order to allow comparisons between T24 and T26 after treatment, Hmin was divided by the resting phase duration (i.e. 14 h and 15 h in T24 and T26, respectively). Two implants in the T24 group and one implant in the T26 group were found partially or totally defective; Tb and LA could not thus be recorded.

(e) Oxidative stress measurements
Mouse lemurs’ blood was collected 3 h before the onset of the individuals. Two hundred microlitre of blood were taken via the saphenous vein, and collected in tubes containing EDTA. Blood samples were then centrifuged at 2000g at 4°C for 30 min and plasma was collected in order to measure plasma 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in ng ml⁻¹ (OxiSelect Oxidative DNA Damage Elisa kit, Cell Biols).

(f) Cognitive apparatus
The cognitive task was first described by Picq et al. [41], inspired from apparatus designed by Lashley [66] for rodents and based on visual discrimination. In the present study, it was conducted over a 2-day period, 3 h before the light extinction. The first day is dedicated to animals’ habituation to the set-up. The discrimination test takes place on the second day. The animal is introduced in a big squared vertical cage through an opening in the wall, to an elevated starting platform. It has to jump onto one of two landing platforms, fixed below on the opposite wall. A hole centred behind the two landing platforms leads to a nesting box behind the cage. On each landing platform, a visual stimulus helps discriminating the left and right platforms. One of these visual clues is the positive stimulus, the other is the negative one. At each trial, the landing platform with the positive clue is kept fixed and leads to the nesting box, whereas the platform with the negative cue is mobile and toggles when the animal jumps on it, such a way that it falls on a cushion pillow, to prevent any injury. At each trial, the location of the positive and negative stimuli on the right or left landing platform is randomized. The animal was given 30 trials to reach the success criterion consisting in 8 jumps on the positive platform within 10 consecutive trials. For each animal, we measured the number of trials needed to reach the success criterion (for more details, see [41]).

(g) Statistical analysis
Normality was verified using Shapiro–Wilk tests. Non-normally distributed data were log-transformed (i.e. LA during the active phase). Outliers were detected and removed using Dixon tests (one T26 individual for oxidative stress). T-tests were used to verify entrainment of T24 and T26 animals. Two-way ANOVAs with repeated measurements (with individuals’ identity as random variable) were performed for the mean VO₂, VCO₂, heat, Tb and LA during active phase and resting phase, and for phase angle, body mass and oxidative stress to assess the effects of treatment (T24 or T26) and time (before and after treatment) simultaneously. A non-parametric Kruskal–Wallis test was applied to compare cognitive performances between the two treatments. Data were analysed using R Studio software with p < 0.05 taken as statistical significance. Data are presented as mean ± s.e.m. Main results are summarized in electronic supplementary material, table S1.

Ethics. All mouse lemurs studied were males born in the laboratory breeding colony of the CNRS/MNHN in Bruxy, France (UMR 7179 CNRS/MNHN; European Institutions Agreement no. E91–114.1). All experiments were performed in accordance with the Principles of Laboratory Animal Care (National Institutes of Health publication 86–23, revised 1985) and the European Communities Council Directive (86/609/EEC). The research was conducted under the approval of the Cuvier Ethical Committee (Committee number 68 of the Comité National de Réflexion Ethique sur l’Expérimentation Animale) under authorization number 12992-2018011613568518 v4.

Data accessibility. The data included in this manuscript are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.jsq75m9v [67].

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