

## REVIEW

# The flexible clock: predictive and reactive homeostasis, energy balance and the circadian regulation of sleep–wake timing

Sjaak J. Riede, Vincent van der Vinne\* and Roelof A. Hut<sup>‡</sup>**ABSTRACT**

The Darwinian fitness of mammals living in a rhythmic environment depends on endogenous daily (circadian) rhythms in behavior and physiology. Here, we discuss the mechanisms underlying the circadian regulation of physiology and behavior in mammals. We also review recent efforts to understand circadian flexibility, such as how the phase of activity and rest is altered depending on the encountered environment. We explain why shifting activity to the day is an adaptive strategy to cope with energetic challenges and show how this can reduce thermoregulatory costs. A framework is provided to make predictions about the optimal timing of activity and rest of non-model species for a wide range of habitats. This Review illustrates how the timing of daily rhythms is reciprocally linked to energy homeostasis, and it highlights the importance of this link in understanding daily rhythms in physiology and behavior.

**KEY WORDS:** Circadian rhythm flexibility, Adaptation, Peripheral clock phase, Energy homeostasis, Circadian thermo-energetics

**Introduction**

The mammalian circadian system is a collection of intrinsic timekeeping mechanisms, differentially regulating biological processes depending on the time of day. The circadian system plays an important role in the variation of daily homeostatic set-points, including fluctuations in plasma glucose, body temperature ( $T_b$ ) and hormone levels (termed rheostasis; see Mrosovsky, 1990). Furthermore, it allows animals to organize their behavior and physiology in anticipation of expected risks and opportunities in their environment and to anticipate predicted perturbations of their internal state (also referred to as predictive homeostasis; see Glossary; Moore-Ede, 1986). To drive these rhythms, most cells possess molecular clocks of interacting ‘clock’ genes that are expressed in an alternating manner. Besides their capacity to promote or inhibit each other’s expression and function, these clock genes also regulate the expression of various tissue-specific genes – referred to as clock-controlled genes (Storch et al., 2002) – thereby regulating tissue activity in a time-of-day-dependent manner. The current consensus is that, in order to produce functional anticipatory rhythms, cells within a tissue have to work together (adjust their timing relative to the timing of other cells) as well as obtain the correct alignment to their (extracellular and extra-organismal) environment. To do so, a light-sensitive master clock in the suprachiasmatic nucleus (SCN) in the hypothalamus sends out

signals that can be used to convey time-of-day information to local tissue clocks or to induce behavioral rhythms at a stable phase relative to the environmental solar day (Dibner et al., 2010, and references therein).

Manipulations such as time-restricted feeding, forced activity and certain (bio)chemical signals can alter the phase of output rhythms relative to that of the light–dark-tracking SCN. As these manipulations also induce self-sustaining rhythms in SCN-lesioned animals, they are considered to be partly or fully reliant on alternative circadian pacemakers (for more detail, see Pezuk et al., 2010). Despite many attempts, the (neuro)anatomical locations and mechanisms of these alternative circadian oscillators have not been identified. It has been proposed that these alternative pacemakers might actually be the result of a network feature of multiple hypothalamic clocks, possibly interacting with local tissue clocks (Acosta-Galvan et al., 2011; Blum et al., 2012; Buijs and Kalsbeek, 2001).

Although we do not fully comprehend how, the organization of circadian rhythms in animals living in the wild is evidently much more flexible than the well-characterized rhythms of laboratory-housed animals. The phase of output rhythms is not enslaved to the phase of the SCN and light–dark cycles alone, but is capable of adjusting to both the experienced and expected environment. Understanding the nature of the flexible timing of output rhythms relative to the SCN might yield important implications for human shift work, chronotherapy (see Glossary), athletic performance and general well-being and health.

Here, we review recent progress in understanding the adaptive flexibility of circadian rhythms. We discuss the idea that, in animals exposed to the same external Zeitgeber (see Glossary) or timing cue(s), differences in energy balance can lead to different circadian phenotypes in a single individual. The data seem to support a model in which, during these adjustments in circadian phenotype, the SCN continues to guide the timing of output rhythms to the most favorable time of day (in contrast to uncoupling from the SCN with rhythmicity being driven by extra-SCN pacemakers). Here, we evaluate our proposed hypothesis explaining why such responses would be ecologically and evolutionarily relevant (the circadian thermo-energetics hypothesis), and our findings that support this hypothesis. We further highlight that divergent phasing between output rhythms and the SCN does not require external timing cues but can be mediated through alterations in an animal’s homeostatic state. The exposure of nocturnal mice to an environment with a constant low food density, or to constant cool ambient temperature ( $T_{\text{ambient}}$ ), can alter their behavioral, physiological and local tissue clock phases relative to the phase of the SCN. This suggests that the circadian system is not only the facilitator of predictive homeostasis, but that it is itself also subject to homeostatic fine-tuning. As the timing (phase) of circadian output rhythms can be modulated by homeostatic changes, we suggest a novel perspective on the flexibility of circadian rhythm generation. Circadian time-keeping mechanisms enable predictive

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**Glossary****Chronotherapy**

Actively taking the (daily) timing of a treatment into account to optimize the effectiveness and/or reduce adverse side-effects in applied medicine.

**Daily temporal niche**

The portion(s) of the day–night cycle that an animal utilizes to compete for ecological resources such as finding food. For example, an animal can occupy a diurnal (day-active), nocturnal (night-active) or crepuscular (twilight-active) daily temporal niche.

**Defended  $T_b$** 

The temperature range of (parts) the body that is actively maintained through homeostatic principles of heat production or active cooling. This temperature can be different for the rest and active phases.

**Food anticipation**

An increase in alertness,  $T_b$  and physical activity in the time (up to several hours) preceding an expected food stimulus.

**Photic entrainment**

The synchronization of endogenous circadian rhythms (e.g. in the SCN) to the environmental 24 h day–night cycle by light.

**Predictive and reactive homeostasis**

Maintaining a given biological variable by physiological mechanisms close to an optimal set point. Reactive mechanisms restore the variable after it goes outside this desired range. Predictive mechanisms adjust the variable before it is expected to go outside this range.

**Temporal niche switching**

Cases under which an individual changes from one daily temporal phenotype to another in order to maximize fitness. A given temporal niche can be associated with different risks and rewards (e.g. predation and thermogenesis) compared with other temporal niches in the same habitat.

**Zeitgeber**

Rhythmic cues in the environment that are capable of entraining (synchronizing) the rhythm of endogenous circadian oscillators with the outside world. Derived from German, meaning ‘time-giver’.

homeostasis, but the circadian machinery itself can be modulated through principles of reactive homeostasis (see Glossary). This changes the view of the circadian phase from being a rigid response to Zeitgeber stimuli to being an adaptive system that actively reshapes how Zeitgebers are processed depending on homeostatic outcomes and internal states.

**The regulation of daily rhythms in mammals****The SCN coordinates output rhythms**

Daily rhythms in sleep–wake behavior, feeding–fasting cycles, drinking behavior, hormone levels, melatonin synthesis and locomotor activity are examples of circadian output rhythms in mammals. They are endogenously driven, as they persist in constant environments with periods close to 24 h (i.e. they are circadian; meaning ‘about a day’). Lesions of the hypothalamic SCN abolish these rhythms (Eastman et al., 1984; Klein and Moore, 1979; Moore and Eichler, 1972; Stephan and Zucker, 1972), whereas transplanting SCN tissue into arrhythmic hosts reintroduces circadian rhythms with a circadian period equal to that of the donor (Ralph et al., 1990; Sujino et al., 2003). The development of novel methods for labeling, imaging, measuring and manipulating neuronal functions has allowed researchers to develop a detailed understanding of how the SCN generates daily rhythms in neuronal firing patterns and how it can match these in their periodicity and in a fixed phase relative to the environment (Abrahamson and Moore, 2001, and references therein). Individual neurons of the SCN show ~24 h rhythms in their spontaneous firing frequency which, in turn, arise from (and feedback on) self-sustaining molecular feedback

loops of clock gene expression (Colwell, 2011; Jones et al., 2015; Nakamura et al., 2002; Shearman et al., 2000). SCN neurons are highly interconnected and can influence each other’s electrical and molecular rhythmicity (Colwell, 2011; Mohawk and Takahashi, 2011; Van Den Pol and Dudek, 1993). Input derived from rhythmic external cues as well as rhythmic internal signals (feedback) can modulate the molecular and electrophysiological rhythms of SCN neurons, altering the phase, period and waveform of the generated rhythm (see reviews by Evans and Gorman, 2016; Yannielli and Harrington, 2004 for details).

In a natural environment, the rhythm of the SCN is considered to be influenced primarily by the light–dark cycle. First, direct and indirect projections from light-capturing retinal ganglion cells reach neurons of the SCN, modulating their activity (Abrahamson and Moore, 2001; Edelman and Amir, 1999; Fernandez et al., 2016; Michel et al., 2006; Nakamura et al., 2004). Depending on the phase of the receiving SCN neuron, light input can stimulate active firing of the neuron and modulate clock gene expression (Brown and Piggins, 2007; Hamada et al., 2001; but also see Drouyer et al., 2007, highlighting SCN heterogeneity in this response). As a result of cellular communication between SCN neurons, adjusting the phase of individual cells can subsequently induce a shift in the activity of the whole SCN (reviewed by Welsh et al., 2010; Belle, 2015). In short, light perceived around dawn will advance the clock phase, such that the next day cells increase their firing frequency earlier. Conversely, light perceived around dusk will delay the clock phase. Through these principles of photic entrainment (see Glossary), the SCN can serve as an accurate clock that generates an internal representation of the external solar day. SCN neurons are most active during the daytime and relatively few cells fire during the night (Schaap et al., 2003); this is true for both diurnal and nocturnal mammals (Challet, 2007; Hut et al., 2012; Inouye and Kawamura, 1979; Kurumiya and Kawamura, 1988; Sato and Kawamura, 1984). Increasing day length widens the distribution of phases of individual SCN neurons, making the SCN an internal representation of both the time of day and the season (Inagaki et al., 2007; Mrugala et al., 2000; Sosniyenko et al., 2009; Sumova et al., 1995; VanderLeest et al., 2007). Neuronal and paracrine output signals from the SCN are thought to be important cues to sustain, phase and pace cellular rhythms in downstream targets, allowing them to align optimally with each other and with the external world (Bartness et al., 2001; Song and Bartness, 1998). Apart from light-derived stimulation, neuronal NPY and serotonin projections can also provide input to the SCN cells; these are collectively referred to as ‘non-photoc’ inputs (Glass et al., 2010; Meyer-Bernstein et al., 1997; Prosser, 2003). In the absence of a light–dark cycle, non-photoc signals, such as a timed palatable meal or timed access to a running wheel, can be sufficient to entrain the phase of the SCN (Castillo et al., 2004; Edgar and Dement, 1991; Hut et al., 1999; Mendoza et al., 2005). Different types of non-photoc cues can be distinguished, and different non-photoc cues may yield completely different phase-response curves and, hence, different modes of circadian entrainment (for a review, see Wams et al., 2017).

**Local tissue clocks**

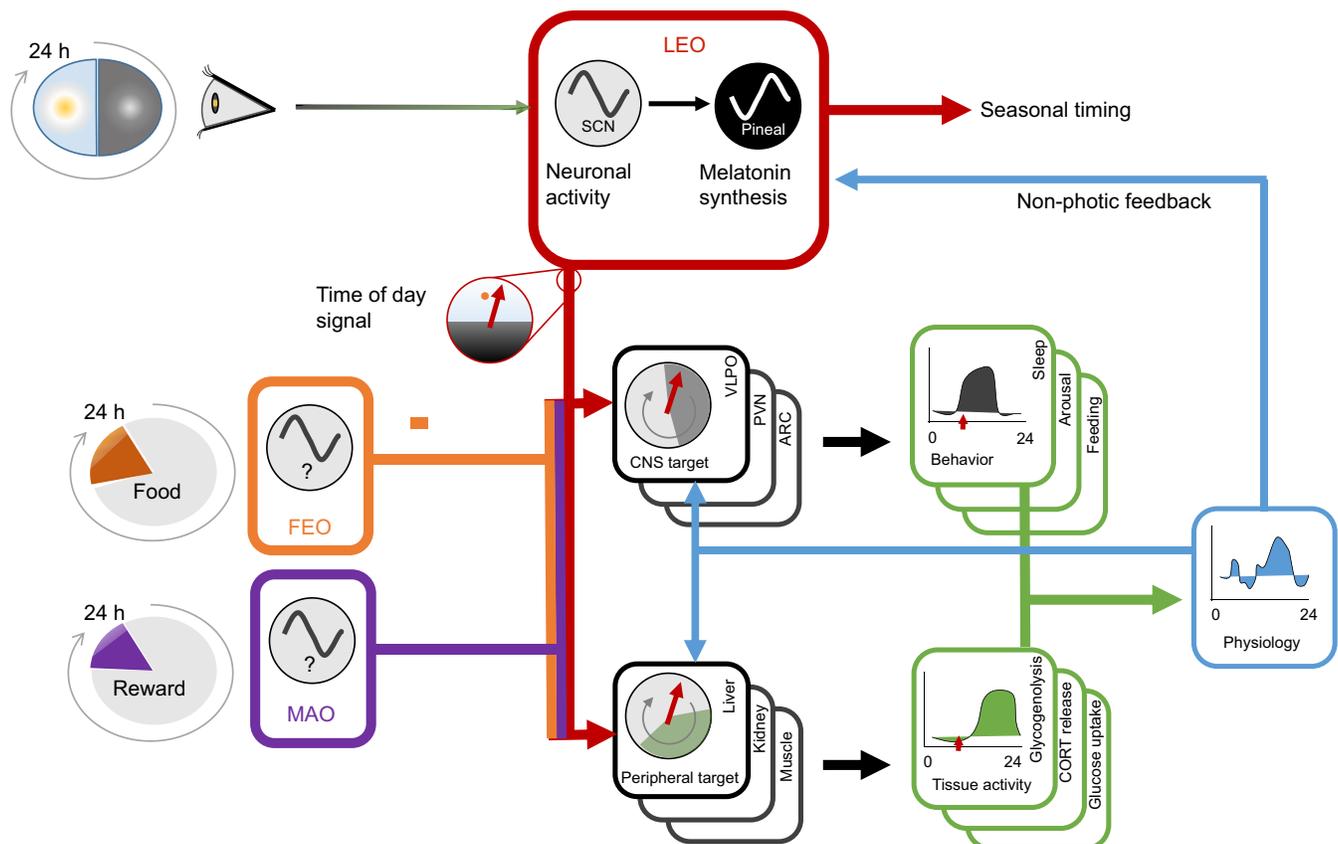
The discovery that clock genes identical or orthologous to those responsible for circadian rhythms in SCN neurons are also expressed in non-SCN cells suggested that most mammalian cells express endogenous circadian rhythms (Balsalobre et al., 1998; Zylka et al., 1998). Instead of having a single well-characterized clock responsible for the control of daily timing (i.e. the SCN), clocks are everywhere in the body. Furthermore, it was shown using

*in vitro* explants that these local tissue clocks can sustain their circadian rhythm in isolation and that they retain rhythmicity in SCN-lesioned animals, albeit with different phases between tissues and with phase differences between animals (Yamazaki et al., 2000; Yoo et al., 2004). Under standardized housing conditions, most peripheral clocks are considered to have a similar phase and bear a stable phase relation to the rhythms produced by the SCN, as shown *in vivo* (Tahara et al., 2012). Lesioning of the SCN results in severely dampened organ-level oscillations, showing the importance of the SCN in maintaining synchrony within peripheral tissues (Tahara et al., 2012), although this was only established for laboratory-housed animals, devoid of natural timing cues. Furthermore, the phase of cellular clocks has been linked to tissue-specific rhythms in gene expression (Storch et al., 2002), giving rise to circadian rhythms in cell function and sensitivity, and leading to rhythmic activation of tissue-specific pathways (Oster et al., 2006; Zhang et al., 2014). The importance of maintaining an appropriate phase of peripheral oscillators is illustrated by the observation that the phase of several local tissue clocks in night-active (nocturnal) species is shifted ~12 h compared with that of

day-active (diurnal) species (Challet, 2007; Hut et al., 2012; Lambert and Weaver, 2006; Ramanathan et al., 2010).

### Phase-control of local tissue clocks by the SCN

As discussed above, light and non-photic feedback cues can set the phase of the circadian rhythm generated by the SCN. The phase of peripheral clocks and the timing of behavioral and physiological output rhythms are controlled through multiple mechanisms. The widely accepted view is that daily rhythms are controlled in a hierarchical manner (summarized in Fig. 1). At the top of this hierarchy is the light-entrainable oscillator (LEO) in the SCN, which generates a time-of-day signal capable of entraining the phase of local tissue clocks both in and outside the CNS (Fig. 1, red box). This time-of-day cue can be transmitted from the SCN via direct neuronal efferent projections (Bartness et al., 2001; Kalsbeek et al., 2004) as well as by diffuse humoral signals (Guo et al., 2005; Silver et al., 1996; Song and Bartness, 1998). It reaches targets in the brain that are responsible for selecting behavior (e.g. sleep, arousal, feeding), as well as peripheral tissues involved in the maintenance of homeostasis. The resulting rhythms in tissue activity and



**Fig. 1. Overview of the proposed hierarchical control of circadian rhythms in mammals.** The high-amplitude endogenous rhythm in the firing rate of suprachiasmatic nucleus (SCN) neurons is entrained to the external light–dark cycle through direct projections of specialized neurons in the retina. The SCN rhythm directly regulates melatonin synthesis in the pineal gland, which, in turn, is involved in seasonal rhythmicity (red box). Together, neuronal and humoral signals from the SCN and melatonin provide the basis for the time-of-day signal to downstream targets in the brain and body (black boxes). The phase of cellular clocks in these targets relates to rhythmic patterns in behavior and tissue activity (green boxes), collectively inducing daily rhythms in physiological variables (blue box). These physiological rhythms, in turn, provide non-photic feedback to both the SCN and local clocks (blue arrows), which can modulate their phase and accuracy. Timed access to food and rewards (indicated by the orange and purple circle segments, respectively) induces rhythmicity in alternative circadian oscillators – the food-entrainable oscillator (FEO) and the methamphetamine-sensitive circadian oscillator (MAO), respectively. Their locations and mechanisms are poorly understood (indicated by the question marks), but they are proposed to guide the circadian phase of CNS and peripheral clocks independent of the SCN phase. The depicted CNS and peripheral targets and their functional output rhythms are chosen as arbitrary examples, and include the arcuate nucleus (ARC), ventrolateral preoptic area (VLPO) and the paraventricular nucleus of the hypothalamus (PVN), the latter linked with rhythmic corticosteroid (CORT) release. LEO, light-entrainable oscillator.

behavior cause rhythmic changes to physiological parameters such as  $T_b$ , heart rate and the levels of circulating nutrients, hormones and metabolites. These physiological rhythms, in turn, can serve as reinforcing feedback to both the SCN (non-photic cues) and to the CNS and local targets (Balsalobre et al., 2000; Buhr et al., 2010; Stokkan et al., 2001; Tataroglu et al., 2015). Under this model, the phase relations between the SCN and peripheral targets are generally regarded as hard-wired and rigid, ensuring the rhythms are accurately timed relative to the light–dark cycle.

#### Phase control of local tissue clocks by non-SCN pacemakers

Non-SCN circadian timing systems can, under certain conditions, determine the phase of local tissue clocks and output rhythms that are otherwise controlled by the SCN (Damiola et al., 2000; Pezuk et al., 2010). Animals with SCN lesions become arrhythmic in constant environments (Eastman et al., 1984; Moore and Eichler, 1972; Stephan and Zucker, 1972). However, timed access to food, reward or methamphetamine infusion can re-introduce daily rhythms in SCN-ablated mammals; alternative (non-SCN) circadian pacemakers have been proposed in order to explain this (Honma et al., 1988; Krieger et al., 1977). The food-entrainable oscillator (FEO) and the methamphetamine-induced circadian oscillator [MAO; also known as the methamphetamine-sensitive circadian oscillator (MASCO); Fig. 1] can induce phase coherence between tissues, as well as allowing (in the case of food) anticipation of daily events that recur at ~24 h periods (reviewed in Mistlberger, 2011). Recently, daily timed exposure to treats, threats and exercise were also shown to induce self-sustaining circadian responses at phases deviating from the SCN-directed rhythm, suggesting that more pacemakers might exist (Flôres et al., 2016; Pellman et al., 2015). The underlying timekeeping mechanisms of non-SCN circadian pacemakers have not been identified (Mistlberger, 2011). However, the dominant view is that non-SCN oscillators either can be slaves to the SCN (thus supporting the robustness of the circadian rhythm) or can become independent in their phase when they receive specific cues, such as methamphetamine or rhythmic food availability (Pezuk et al., 2010).

Most cells and tissues – including many brain regions – possess clock gene cycles and could thus potentially serve as extra-SCN circadian rhythm generators. Multiple interactions between the clocks in different tissues might explain why identifying the locations of extra-SCN pacemakers has proven difficult; many of the involved components may show some degree of redundancy. Hierarchical organization of the communication pathways between the clock gene rhythms in local tissues remains largely unestablished, although it has been shown that various output rhythms (including corticosterone, melatonin and  $T_b$  patterns) can play important roles in the entrainment of circadian oscillations at the tissue level (Balsalobre et al., 2000; Buhr et al., 2010; Torres-Farfan et al., 2011). It is thought that these ‘output-as-input’ interactions provide robustness to the circadian system, but they might also facilitate collective flexibility in the timing of overt rhythms in behavior and physiology relative to the SCN phase. Rhythms in behavior and physiology can thus be regarded as both cause and consequence of peripheral clock phasing (Fig. 1, black and blue arrows).

#### Circadian flexibility: the ‘work-for-food’ paradigm

##### Ecological relevance of circadian flexibility in the phase of daily rhythms

Under 24 h entrainment, circadian rhythms are routines that repeat themselves on a daily basis. The purpose of these routines is twofold: first, they allow animals to prepare and anticipate their

actions, thereby utilizing time and resources more efficiently; second, routines provide safety – ‘if it worked for me yesterday, it will probably work today’. As for most traits found in nature; the performance of routine behavior has emerged under, and has been shaped by, the pressures of natural selection. Thus, the phase of the daily routines is optimized for an animal’s natural habitat. These habitats are often complex and diverse (e.g. arctic versus tropical, desert versus wetland), providing the basis for the large diversity in the phase of the circadian rhythms found in their inhabitants. Generally, some of the environmental parameters relevant for survival of a given species are bound to show daily variation. These can be direct and abiotic (e.g. light, temperature, UV radiation) or indirect and biotic factors (e.g. the presence of conspecifics, prey, predators and competing species). Furthermore, habitats themselves can be dynamic (e.g. seasonal or sudden changes in weather, food and predator density, parasite and disease loads), resulting in differences in the optimal circadian phenotype. Often, animals will have to make trade-offs in their selection of daily temporal niche (see Glossary), as the safest time to forage might coincide with the least efficient time for finding food, for example. Field observations clearly indicate that daily rhythms of free-living animals can differ from their rhythms when kept under laboratory conditions, or can change within individuals in response to the dynamics of their habitat or to their specific priorities (reviewed in Hut et al., 2012). The mechanisms underlying this flexibility are poorly understood, but seem to be regulated by modifiers downstream of the SCN (Kas and Edgar, 1999; van der Vinne et al., 2014b). This line of investigation might eventually bear relevant insights for targeted treatment of humans who are facing frequent disruptions of their daily routines (e.g. shift work, traveling time zones, irregular lifestyles) or who have reduced exposure to natural light–dark cycles (Woelders et al., 2017).

#### Developing the ‘work-for-food’ model to study circadian flexibility

The relevance of the flexible timing of circadian rhythms was apparent when our lab attempted to unite the results of several lines of research involving behavioral energetics, circadian rhythms and ecological timing. Firstly, Daan et al. (2011) found that, for extended periods of time (up to several months), house mice kept under natural conditions in outdoor field enclosures were exclusively active during the day, whereas during other periods they were more nocturnal (Daan et al., 2011). Around the same time, Schubert et al. (2008) performed lab studies on the effect of habitat quality and energy balance on physiological responses in CD1 mice. They compared female mice exposed to high foraging costs (incurred by wheel running) with mice receiving a matched food intake without the need to forage for it. Mice that had to work hard to obtain their food showed reduced daily energy expenditure, had a lower resting metabolic rate and ceased estrous cyclicity. Under these conditions, mice started to show daily torpor – a state in which metabolism is greatly reduced for several hours, resulting in a pronounced drop in  $T_b$  (Schubert et al., 2010).

Schubert’s work inspired Pilorz to use the same set-up to develop a paradigm for simulated shift work, where mice could obtain food rewards only at pre-scheduled times of day. During training, mice could earn food during the dark and their workload per food reward was increased, but, surprisingly, the mice phase-advanced their activity period to the extent that they were resting during the scheduled shift work window. This resulted in reduced foraging yields and dramatic weight loss (Violetta Pilorz, personal communication). We subsequently performed this experiment without time constraints to the reward window (mice could work

for food for the full 24 h cycle), and found that increased workload (i.e. increased traveling distance in the wheel per unit food reward) shifted the activity of CBA/CaJ mice from the night into the day (Hut et al., 2011). With this paradigm, there are no external Zeitgebers provided other than the light–dark cycle. The pre-set workload is equal over the 24 h cycle, and temperature and humidity are tightly controlled at stable values. This ‘work-for-food’ (WFF) protocol induced robust and repeatable temporal niche switching (see Glossary) in response to increased work load – every mouse tested showed a shift from nocturnal to diurnal activity rhythms when the food reward ratio was decreased.

To establish whether the change in circadian organization was related to reduced food intake per se or negative energy balance in general, we tested whether lowering  $T_{\text{ambient}}$  would yield similar effects. Indeed, mice fed *ad libitum* showed a significantly higher proportion of their activity during the light phase at lower  $T_{\text{ambient}}$  (~10% at 25°C and up to 32% at 10°C). In addition, lower  $T_{\text{ambient}}$  enhanced the effect of WFF on diurnality (van der Vinne et al., 2014b). Collectively, these results suggest that negative energy balance, caused either by lowering energy intake or by increasing energy expenditure, favors a diurnal phenotype. Reviewing the available literature indicates that temporal niche switching is widespread in nature, but mainly in predominantly nocturnal mammals, where it often occurs in response to changes in energy consumption or availability (Hut et al., 2012). In summary, factors that have a negative impact on energy balance seem to be associated with increased daytime activity, whereas energy surplus (such as *ad libitum* feeding in the lab) or high daytime temperatures can lead to increased nocturnal activity (Hut et al., 2012).

### Functional consequences of flexible circadian phenotype: the circadian thermo-energetics hypothesis

#### Benefits of flexible output rhythms: a diurnal lifestyle reduces energetic needs

The adaptive significance of temporal niche switching from nocturnal to diurnal activity may involve a reduction in energy consumption. This is the basis of the circadian thermo-energetics (CTE) hypothesis, which builds on two basic features: (1) the night is colder than the day, and (2) sleep and rest are associated with better thermal insulation and therefore reduced heat loss (Hut et al., 2012). Examples of such strategies preventing heat loss include postural changes, reduced breathing rate, reduced blood flow to (and temperature of) the extremities, huddling with conspecifics or sleeping in an insulated temperature-buffered location such as a burrow or nest (see Table 1). Because thermal insulation reduces a fraction of the energetic costs required to maintain  $T_b$ , it will return higher energy savings in absolute terms at low  $T_{\text{ambient}}$ . Aligning the rest phase with the night and actively foraging during the day is therefore more energy efficient under most natural conditions (van der Vinne et al., 2015a). Nocturnality is therefore energetically costly, as discussed below.

#### Burrowing animals: diurnality provides energetic benefits by altering daily ambient temperature exposure

$T_{\text{ambient}}$  influences the energy expenditure of endothermic animals because it affects the rate of heat loss and, thus, thermoregulation costs. Daily patterns in surface temperature ( $T_{\text{surface}}$ ) are common in many habitats, with temperature peaking during the daytime and being lowest at night (Fig. 2A;  $\Delta$ ). Many species are not continuously exposed to the  $T_{\text{surface}}$  cycle, but instead retreat into the safety and thermal comfort of a burrow when resting. Burrows are buffered from the  $T_{\text{surface}}$ , having a reduced daily amplitude of

**Table 1. Variables that determine steepness of Scholander slopes**

Variable	Phase	Effect on slope
Insulation material around the body	Rest	Decrease
Huddling with conspecifics	Rest	Decrease
Avoiding wind chill (sheltered location)	Rest	↓
Vasoconstriction in periphery	Rest	↓
Reduced surface area (curled up sleeping postures)	Rest	↓
Low maintenance costs of posture (e.g. lying down)	Rest	↓
Torpor*	Rest	←/↓
Wind chill	Active	↑
Humidity and rain	Active	↑
Vasodilation in periphery	Active	↑
Higher breathing rate	Active	↑
Activity-related thermogenesis	Active	↓
Sun basking <sup>‡</sup>	Active	↓
Increased subcutaneous fat mass	Active/ rest	↓
Increased fur thickness	Active/ rest	↓
Digestion-related thermogenesis	Active/ rest	↓
Increased body size	Active/ rest	↓

\*It is debated whether torpor can be seen as resting, but it does rule out physical activity. As it is a severe reduction of the defended  $T_b$ , it causes a leftward shift of the Scholander curve, often bringing the  $T_{\text{LC}}$  to below  $T_{\text{ambient}}$ , thereby reducing thermogenesis to very low values. The net result is lower energy expenditure at the same  $T_{\text{ambient}}$ .

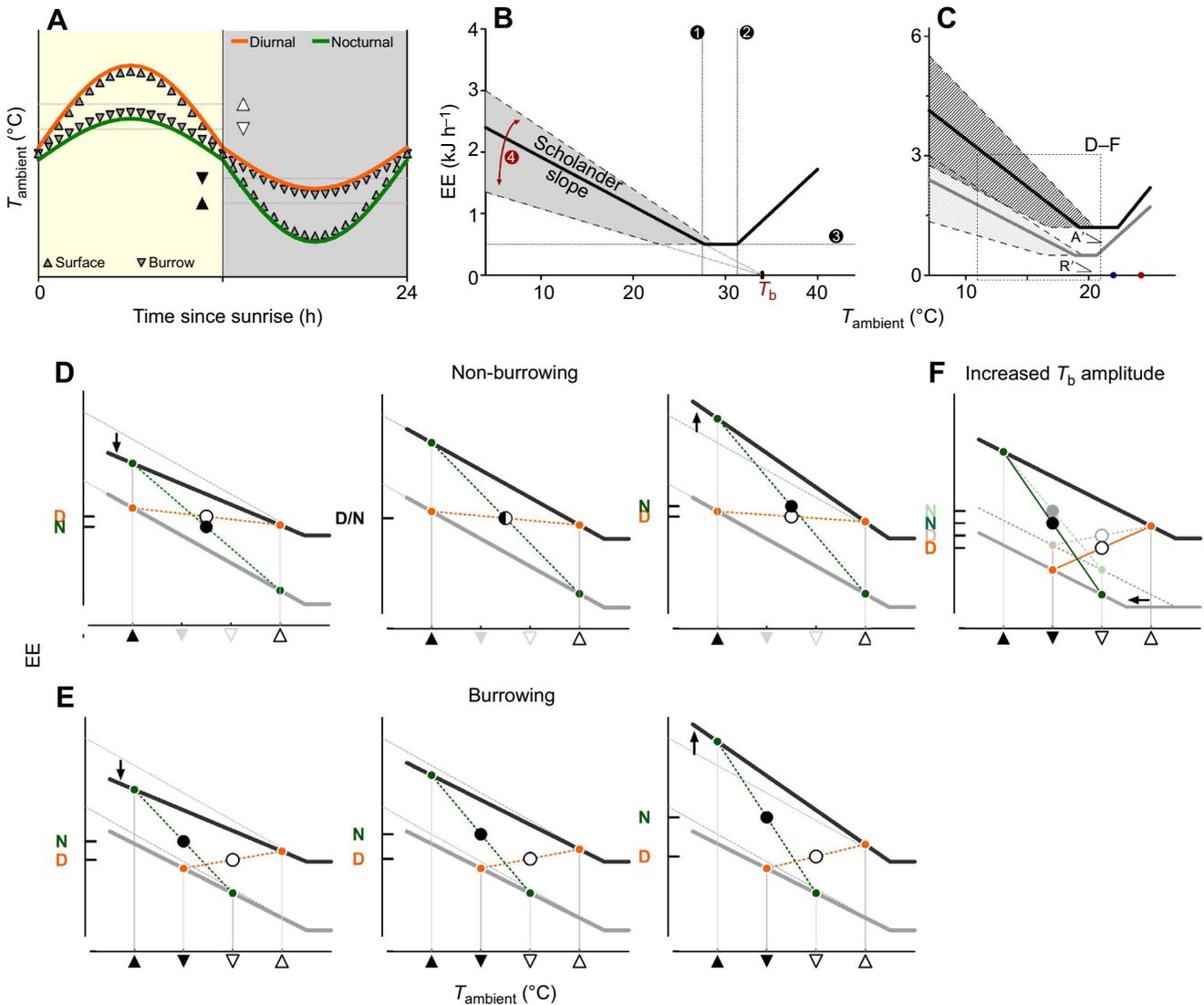
<sup>‡</sup>Sun basking is only available during the daytime and on the surface. As resting in such an exposed location is dangerous for many prey species, it is most commonly utilized in the active phase. However, some species (e.g. larger carnivores) might also utilize sun basking in their rest phase.

temperature change and – in some cases – a slightly delayed phase (Fig. 2A;  $\nabla$ ). Retreating into a burrow at night provides an energetic benefit, as burrowing animals will encounter a higher average 24 h temperature (Fig. 2A, red curve) compared with when they are active during the cold night and rest in their burrow during the daytime (Fig. 2A, green curve).

In our example (Fig. 2A), the mean burrow temperature ( $T_{\text{burrow}}$ ) falls within the  $T_{\text{surface}}$  range. It is conceivable that in some ecological habitats this is not always the case. For example, the mean  $T_{\text{burrow}}$  of an arctic ground squirrel might be low and fairly constant in a frozen tundra, well below the daily average of the air temperature (Long et al., 2005). Conversely, the mean 24 h  $T_{\text{burrow}}$  temperature in a dark-soiled rocky location, or near geothermally active sites, might exceed the average 24 h air temperature (e.g. see Tomotani et al., 2012). However, a burrowing animal would also spend less energy on thermoregulation under these conditions when diurnal, as long as  $T_{\text{burrow}}$  and  $T_{\text{surface}}$  fall below the animals’ thermoneutral zone (TNZ; see below). Provided that the animal needs to surface for foraging, aligning this activity with the warmest  $T_{\text{surface}}$  is energetically favorable as long as the  $T_{\text{burrow}}$  amplitude is lower than the  $T_{\text{surface}}$  amplitude. Conversely, when  $T_{\text{ambient}}$  exceeds the TNZ of an animal, as might be the case in hot climates, predictions are that activity is better avoided in the warmest part of the day to conserve energy.

#### Relationship between ambient temperature and energy expenditure

The relationship between energy consumption and  $T_{\text{ambient}}$  is described by Scholander curves (Scholander et al., 1950; Fig. 2B). Endothermic animals actively maintain their  $T_b$ , investing energy to



**Fig. 2. The energetic benefit of diurnality depends on the encountered temperature profile and the difference between the rest and active phase Scholander slopes.** (A) Surface ( $\Delta$ ) and burrow ( $\nabla$ ) temperatures across the solar day are modeled by a sinusoidal pattern, with the amplitude difference depending on the insulation constant of the burrow. Diurnal burrowing animals (orange line) encounter higher ambient temperatures than nocturnal animals (green line). The larger triangles represent the mean temperatures for daytime (when white) and night-time (when black) for surface ( $\Delta$ ) and burrow ( $\nabla$ ), respectively. The light–dark cycle is represented by the background colors (yellow for daytime, gray for night). (B) As a result of thermoregulation costs, mammals increase their energy expenditure to above their thermoneutral resting metabolic rate (3) at temperatures further removed from their thermoneutral zone [below the lower critical temperature (1) and above the upper critical temperature (2)]. The Scholander slope depends on heat conductance and (4) can be modulated by physiological, anatomical and behavioral adaptations (indicated by the gray shaded zone). The Scholander slope intersects the horizontal axis at the defended body temperature ( $T_b$ ). (C) During rest and active phases, differences in defended  $T_b$  (blue and red dots, respectively) and physical/physiological activity mean that the rest phase and active phase are classified by separate slopes (R' and A') and different basal rates of energy expenditure (horizontal plateaus). (D) Without a burrow, the phase with the lowest slope (resting or active, represented by gray and black lines, respectively) is best performed at night, in order to conserve energy. (E) Burrows, by changing the encountered temperature, reduce the energy expenditure when they are utilized during the cold night. In addition, they reduce the rest-phase Scholander slope. (F) Besides the slopes, TNZ metabolic rates and defended  $T_b$  also have an impact on energy expenditure. However, these horizontal or vertical shifts in one or both of the curves do not in themselves favor diurnal or nocturnal behavior per se, as the absolute difference in energy expenditure between a diurnal and nocturnal circadian phenotype is maintained. The graph in F shows an example where the defended  $T_b$  is reduced during the rest phase. Plots are stylized for clarity but rely on measures of energy consumption of mice kept under various ambient temperatures and housing conditions (original data in van der Vinne et al., 2014b, 2015b). The x-axis triangle symbols in D–F are explained by figure and text of A. The colored 'D' and 'N' reflect the mean hourly energy consumption for fully diurnal (open circle) and nocturnal (closed circle) animals under the indicated condition (equal rest and active phase duration).

generate heat or to lose it by panting and sweating. The range of temperatures where no energy investment is needed for thermoregulation is called the TNZ; in this temperature range, energy expenditure is considered nearly constant. The borders of the

TNZ are called the lower and upper critical temperature ( $T_{LC}$  and  $T_{UC}$ , respectively) (Fig. 2B). For each degree away from the thermoneutral border (below  $T_{LC}$  or above  $T_{UC}$ ), energy expenditure increases in a linear fashion (the 'Scholander slopes'), representing

higher thermoregulation costs (Scholander et al., 1950). When the  $T_{\text{ambient}}$  falls below  $T_{\text{LC}}$ , metabolic rate increases so that heat produced by the body matches the heat loss to the environment, thereby maintaining  $T_b$ . The TNZ metabolic rate, defended  $T_b$  (see Glossary) and heat dissipation rate of an animal are generally different for the active and rest phases. For example, reduced blood flow towards skin and limbs during resting will decrease heat dissipation from the skin to surrounding air, reducing the Scholander slope (Fig. 2B and Fig. 4). Therefore, the Scholander curves of the active (A') and resting (R') phases are different in shape (Fig. 2C).

#### Differences in the active and resting phase Scholander slope predict energetic costs of diurnal versus nocturnal rest–activity rhythms

Numerous behavioral, anatomical and physiological adaptations play a role in reducing energetic costs by reducing conductance (the rate of heat loss per degree of difference between  $T_b$  and  $T_{\text{ambient}}$ ). Despite this, for small endothermic mammals facing naturally occurring low temperatures, thermogenesis costs can comprise more than two-thirds of their daily energy budget (Chappell et al., 2004). Any adjustment in temporal organization of rest and activity that reduces these costs could therefore be an ecologically relevant strategy for survival when facing adverse energetic circumstances (e.g. food scarcity or cold weather). The slope of the Scholander curves of the rest and active phase can be modulated by numerous external and internal variables for animals living in their natural habitat (Table 1). For instance, social animals might reduce the steepness of the rest phase slope by huddling to share body heat during their rest phase (Fleming, 1980). The steepness of the active phase slope might be reduced by utilizing sunlight to warm the body (sun basking; Geiser et al., 2016) or utilizing the heat generated by physical activity for thermogenesis (Weinert and Waterhouse, 1998). Conversely, exposure to wind and rain steepens slope A' significantly. An overview of some relevant variables, and how they influence the slopes (A', R', or both) is provided in Table 1. Some of these variables might not be relevant for all species, whereas additional unmentioned variables might apply to others. Because of these influences, the rest and active phase slopes can differ in steepness. As illustrated in Fig. 2, in order to conserve energy, it would be advantageous to perform the behavioral phase (either activity or rest) associated with the lowest steepness during the coldest part of the daily  $T_{\text{ambient}}$  cycle (Fig. 2D). These effects add to the energetic effects of altered  $T_{\text{ambient}}$  exposure by burrowing (Fig. 2E), as discussed above.

#### Body temperature amplitude and TNZ metabolic rates have an impact on energy expenditure, but do not favor diurnal or nocturnal behavior in itself

An additional strategy used by mammals to reduce energy expenditure when facing harsh conditions might be to reduce their metabolic rate by reducing their investments in immune functions or reproduction (Martin et al., 2008), or by reducing their defended  $T_b$  during the rest phase (Yoda et al., 2000). These responses will cause a vertical or horizontal shift of one or both of the Scholander curves. However, as long as these shifts have no influence on the steepness of the slopes, and the ambient temperature remains below the  $T_{\text{LC}}$ , they will not favor diurnal or nocturnal behavior per se (illustrated in Fig. 2F). The absolute difference between a nocturnal and diurnal phenotype in response to these shifts stays constant. This means they can alter the size of a relative benefit (e.g. diurnal energy expenditure might change from 75% to 60% in relation to nocturnal energy expenditure), but never the direction. In summary, the slopes

(R' and A') and the  $T_{\text{ambient}}$  to which an animal is exposed determine the energetically optimal phase for activity.

#### Energy expenditure measures confirm that diurnality in mice would save energy in their natural habitat

Recently, we used an indirect calorimetry set-up to assess the minimal hourly energy expenditure in resting and active mice (CBA-CaJ strain) for a range of temperatures in and below the TNZ (10–30°C), and constructed the Scholander curves of their rest and active phases (van der Vinne et al., 2015b). Additionally, we measured the resting phase energy consumption for the same range of temperatures when mice were given increasing amounts of nest material (cotton wool) or cage partners to allow huddling (in groups of 1–3 mice) during rest. To estimate the effect of activity-related thermogenesis, we measured energy expenditure during the active phase both with and without access to a running wheel. To incorporate the effect of wind chill, we compared the rate of heat loss of a warmed-up dead mouse in both the open field (in shade) and indoors. To incorporate the temperature-buffering capacity of a burrow, we simultaneously measured  $T_{\text{surface}}$  rhythms and the temperature rhythm in a hay-filled nest box. Additional measures regarding the thermal buffering capacity of natural nests and burrows were obtained from the literature. Lastly, we measured  $T_b$  and energy expenditure during the resting and active phase for mice that were working for their food and were thus required to minimize their energy expenditure (van der Vinne et al., 2014b). Combining these measurements, we showed that mice can indeed reduce their daily energy expenditure by adopting a diurnal phenotype. By incorporating a buffered nest temperature cycle and the thermal benefit of nesting (huddling and insulation) into the rest phase Scholander curve, and considering the effect of wind chill on the active phase Scholander curve, we calculated that there is a steeper slope for the active phase than for the resting phase Scholander curve. This provides a situation where diurnality reduces energy expenditure (Fig. 2E, right plot). We estimate that diurnality in mice would reduce their total daily energy consumption by ~6–10%. This does not include the energy-saving effect of torpor at the end of the night (the coldest phase of the day), which would reduce their total daily energy consumption by an additional ~10%. Differences in the amplitude and shape of the  $T_{\text{surface}}$  curve, as well as differences in the duration of the active phase of the mice can reduce or increase this energetic benefit. Projecting our model data to a wide range of habitats – utilizing hourly temperature data from across Europe – the diurnal benefit holds for all geographical locations with daily  $T_{\text{surface}}$  rhythms that are below the TNZ of the species (van der Vinne et al., 2015a). Furthermore, it is very consistent over days; a diurnal benefit would be present on 98.5% of all individual days within a single location (van der Vinne et al., 2015a). The fact that a diurnal phenotype reliably provides energetic savings relies on thermodynamics and would therefore extend to other mammals, but the precise magnitude of energetic savings would depend on factors such as individual lifestyle, specific habitat characteristics and body size.

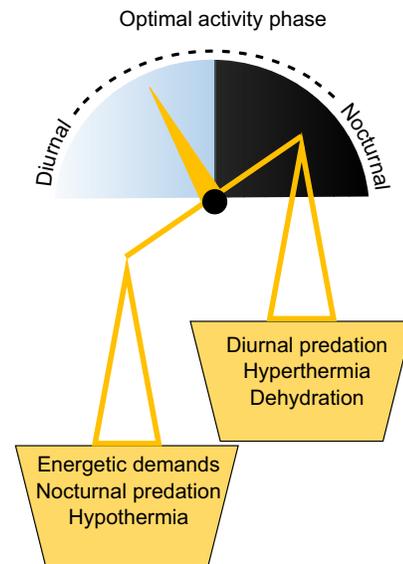
#### Diurnality: cheap but risky?

In nature, animals face important trade-offs to ensure survival and successful reproduction. Obtaining sufficient resources whilst guarding against pathogens, injury and predators is often essential for increasing fitness. As small endothermic burrowing mammals require a lower energy intake when they exhibit a diurnal phenotype, the question arises as to why many small mammals are nocturnal. First, we would like to stress that, in their natural habitat, many small rodent species typically considered as nocturnal can indeed show

extensive periods in which a substantial or even the dominant fraction of their daily activity occurs during the light phase. This includes rats, mice, hamsters and even several subterranean species (Daan et al., 2011; Gattermann et al., 2008; Harper and Bunbury, 2015; Levy et al., 2007; Tomotani et al., 2012; Urrejola et al., 2005; reviewed in Hut et al., 2012). We propose that the danger of predation might be an important factor in favoring nocturnality in rodents and other herbivorous prey species whenever they can afford it. Indeed, some studies report high amounts of diurnal activity in rats on islands devoid of natural predators (e.g. Harper and Bunbury, 2015). Diurnality in these rats seems especially high in years with high population densities when food becomes scarce, which would indeed increase the pressure to reduce energy expenditure. Additionally, some evidence suggests that prey species can actively alter their activity patterns in response to changes in the perceived nocturnal or diurnal predation risk (Bakker et al., 2005; Fenn and Macdonald, 1995). Conversely, black bears, which lack natural (non-human) predators and are largely herbivorous (85% of their diet consists of plant material), are generally diurnal, but can alter the timing of their activity during the hunting season, when bears show more movement and road-crossing behavior during the night when hunters are absent (Stillfried et al., 2015). It is hard to measure and compare the relative predation risks in a certain habitat for both day and night. Some insights can be acquired indirectly by observing vigilance levels (time and effort expended to monitor the environment for danger). Variation between species and habitats is large when it comes to diurnal and nocturnal predation risks, but in most species that forage both during the day and the night, vigilance levels appear higher during the daytime, suggesting that the night might be the safer daily temporal niche for foraging (Beauchamp, 2007). In addition to high diurnal predation risk, the risk of overheating when daytime temperatures approach or exceed  $T_{UC}$  (van der Vinne et al., 2014a) or the risk of dehydration (Levy et al., 2016) might facilitate nocturnality in some habitats (Fig. 3). For some species, the risk of UV damage to the eyes or skin might also prevent daytime activity (Hut et al., 2012). Ultimately, the best time of day to become active is the phase that optimizes the benefit-to-cost ratio of all fitness components.

#### Circadian flexibility is regulated by robust and flexible clocks

In mice, energetic challenges result in a phase advance of behavior and most physiological rhythms ( $T_b$ , food intake, plasma corticosterone, peripheral clock gene expression), while the SCN phase remains unaltered (Hut et al., 2011; van der Vinne et al., 2014b). The experiments leading to this conclusion were performed under standard laboratory conditions (stable  $T_{ambient}$ , no burrowing, no predation) where light–dark cues were the only available Zeitgeber. Subjecting mice to phase shifts of the light–dark cycle revealed that the activity of mice remained entrained to this cycle (van der Vinne et al., 2014b). When exposed to competing Zeitgeber signals (a 10°C temperature cycle that was in anti-phase with light–dark cues), mice also remained entrained to the light–dark cycle (van der Vinne et al., 2014b). In the absence of all timing cues in constant darkness, a main bout of locomotor activity appeared to free-run with a constant period, while a shifting activity bout phase-advanced during the energetic challenge of the workload regime (Hut et al., 2011). Collectively, these observations show that: (1) the SCN remains entrained to the external light–dark cycle under energetically challenging conditions; and (2) the phase of activity and peripheral clocks shift around the timing information provided by the SCN (and light–dark cycle), depending on an animal's energetic state.

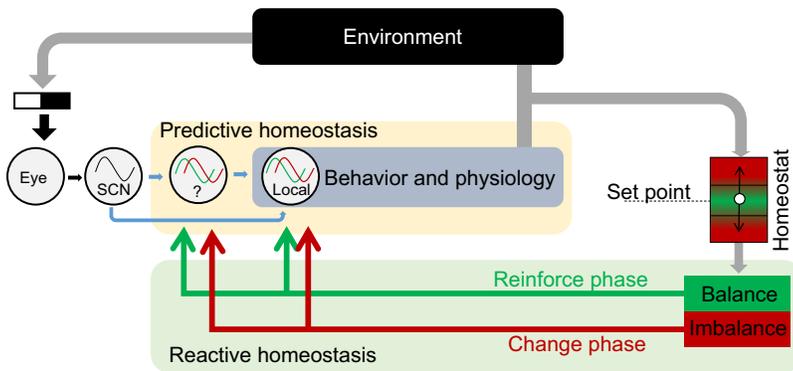


**Fig. 3. The optimal activity phase.** The optimal circadian phenotype is the outcome of weighing multiple aspects of the habitat that pose risks and opportunities relevant to an animal's fitness. The optimal phenotype will differ depending on the species or habitat in question. For example, a high risk of daytime predation would favor a nocturnal phenotype, whereas the risk of hypothermia would favor diurnality.

Although the benefit of diurnal activity in small nocturnal mammals is to reduce thermogenesis costs, shifting physiology and behavior to the light phase will also occur when energetic benefits are absent (e.g. when  $T_{ambient}$  is constant or in anti-phase; van der Vinne et al., 2014b). Under natural conditions, weather changes that similarly result in reduced energetic benefits of diurnality rarely occur (van der Vinne et al., 2015b). Conversely, clocks facilitate the anticipation of frequent (and therefore predictable) conditions. The high probability that daytime temperatures will be higher than night-time temperatures explains why the SCN, entrained by the light–dark cycle, is used as a stable and reliable proximate cue to predict the energetically optimal phase of activity. The flexible phase of output rhythms is thus always influenced by the stable (entrained) phase of the SCN, to ensure that the flexible circadian phenotype can be adjusted to different (optimal) phases of the external day. This novel view, that extra-SCN oscillators are flexibly coupled to the SCN phase, collectively determining output rhythms, is gaining more support (Pendergast and Yamazaki, 2014). Experiments aiming to isolate the role of the SCN in regulating this flexible circadian phenotype in mice are currently ongoing.

#### Homeostatic feedback on peripheral circadian phase

Alternative (extra-SCN) circadian pacemakers such as the FEO are often considered to rely on environmental timing cues to produce a certain (novel) phase. In our experiments, such timing cues are absent, revealing that the change to diurnality is endogenous. In free-living animals, food intake is often the result of foraging activity, and mechanisms underlying food entrainment might naturally emerge when foraging success is high at specific times of day. However, running that is rewarded with food is not sufficient to induce diurnality: mice that can earn palatable chocolate-flavored pellets with running (whilst being fed with *ad libitum* chow) do not shift their circadian phase (van der Vinne et al., 2015b). Similarly, working for food at low workloads (small distance of running per pellet) does not induce significant changes to the circadian phase of



**Fig. 4. Illustration of how circadian clocks allow predictive homeostasis and receive reactive homeostatic feedback.** Daily rhythms in behavior and physiology are organized by local circadian clocks ('Local'), present in most cells of the body. These rhythms are timed through input directly from the SCN, potentially via intermediate pacemakers (denoted by '?'; these could be synonymous with FEO and MAO in Fig. 1). The interactions of behavior and physiology (internal state) with the environment (external state) determine how well the animal can maintain homeostasis. If balance in homeostasis can be maintained, the phase angle between the SCN and downstream targets is reinforced, whereas an imbalance promotes phase changes, leading to circadian flexibility.

the rest–activity rhythm (Hut et al., 2011). Weight loss over multiple days seems to be necessary before the diurnal phenotype emerges but, once induced, the diurnality can be maintained in mice with stable body mass (ranging from ~18 to >30 g, suggesting that absolute body mass might be of lesser importance). Daily torpor does occur in some, but not all, shifted mice, and the advance of the circadian phenotype always precedes the occurrence of the first daily torpor, making it an unlikely component of the mechanism to induce a diurnal phenotype (Hut et al., 2011). Anatomically, the shifted mice show reduced deposits of white adipose tissue, both abdominally and subcutaneously. Male mice have reduced testes size and lower basal plasma glucose levels (R.A.H., Violetta Pilorz, Willem Eijer and S.J.R., unpublished data), whereas their corticosterone levels are higher than those of non-shifted males (van der Vinne et al., 2014b). These alterations to homeostatic parameters might provide a signal to change the phase alignment of extra-SCN clock gene cycles in relation to the SCN. Glucose sensing in particular might be an integral component of circadian phase responses to prolonged reduced food intake (Challet et al., 1998). Hypoglycemia induces the release of adrenalin and alters circulating insulin levels, both of which modulate the phase of peripheral clock gene rhythms (Terazono et al., 2003). Inducing hyperglycemia without increasing body mass – by feeding mice a diet with a very high fat content – was shown to delay the clock in peripheral tissues and to phase-delay rest–activity rhythms (Genzer et al., 2015; Honma et al., 2016). Furthermore, recently it was reported that liver-derived ketone bodies (resulting from conversion of endogenous fat reserves to supply energy) play an important role in the rhythmicity of food anticipation (see Glossary; Chavan et al., 2016). Besides these energy homeostasis-related variables, the responses to hypoxia, hyperthermia, hypothermia, inflammation and other cellular stressors also provide feedback to (or modulate) the phase of peripheral clock gene expression rhythms (Cavadini et al., 2007; DeBruyne et al., 2015; Gerber et al., 2015; Tamaru and Ikeda, 2016). Collectively, these findings point towards a system in which failure to maintain physiological variables within their usual homeostatic range can alter the circadian timing of output rhythms.

To maximize fitness, animals must optimally interact with their environment in order to maintain homeostatic balance over a prolonged timespan. The temporal organization of physiology and behavior are key to successfully maintaining this balance. We argue here that the circadian timing of output rhythms (peripheral clocks regulating cellular activity and behaviors) is reciprocally linked to homeostasis. If the phenotype is successful (i.e. 'in balance'), it strengthens the daily routines, leading to a robust phase. By contrast, prolonged failure to maintain homeostatic balance can alter the phase-angle between peripheral clocks and the SCN (Fig. 4). Flexibility in output rhythm timing seems to be an essential feature

of the circadian system, allowing it to cope with variations in the quality or temporal dynamics of the habitat.

### Factors influencing circadian niche in the wild

#### Field data indicates that circadian niche depends on context

Our circadian thermo-energetics hypothesis aims to explain the relevance of temporal niche switching; connecting energy balance, circadian timing and predation risks. Recently, Stawski et al. (2016) studied the effects of a forest fire on the behavior and thermal rhythms of wild brown antechinus (*Antechinus stuartii*), a small insectivorous Australian marsupial mammal living in densely vegetated forests. When fires destroy much of their habitat these animals survive by retreating into burrows or rock crevices, but are subsequently faced with a harsh and dangerous environment with little cover, increased predation pressure and low food abundance. In response, they reduce their activity levels after the fire and show reduced daytime foraging (proposed to alleviate their predation risk), which is energetically compensated for by spending more time in daily torpor. Similarly, nocturnal gray mouse lemurs (*Microcebus murinus*) spend a larger portion of the night in torpor when they are energetically challenged in a controlled laboratory experiment (Canale et al., 2011), which can thus advance the timing of foraging behavior, in some cases forcing it into the day.

The opposite response, temporal niche switching of diurnal species towards nocturnality, is also not uncommon. Some rodent species that are diurnal in the field become nocturnal under certain laboratory conditions, including degus (Ebensperger et al., 2004; Kas and Edgar, 1999), hamsters (Gattermann et al., 2008), tuco-tucos (Tomotani et al., 2012), cururos (Urrejola et al., 2005), Nile grass rats (Blanchong et al., 1999) and golden spiny mice (Cohen et al., 2010). These discrepancies in circadian rhythms between the laboratory and field raise the question of whether a 'default' circadian phenotype actually exists. Does the animals' natural habitat present constant threats or challenges that drive them to become diurnal (e.g. low food abundance, lack of water) or are the unnatural laboratory conditions changing the phase of entrainment? Regardless, these examples point out that circadian phasing of daily rhythms can be flexibly aligned based on the context and quality of the habitat or state of the animal.

#### Food, water and shelter can modulate circadian phase

To further exemplify the complexity of the circadian niche, fat-tailed sand rats (*Psammodmys obesus*), which live in arid desert conditions, are diurnal in the field but show a nocturnal phase of general activity and peak  $T_b$  in the lab when housed with *ad libitum* food (Barak and Kronfeld-Schor, 2013). Unlike wild animals, captive animals are not exposed to UV radiation, lack a natural daily fluctuation in  $T_{ambient}$  and humidity, and often have *ad libitum* access to food and water. In

addition, laboratory-housed animals generally lack the opportunity to burrow. Burrows provide the animal with a safe shelter, and a lack thereof might increase anxiety, potentially contributing to the shift in activity to the night, which might be perceived as safer. In addition, burrows can have a strong effect on evaporative water loss, another abiotic factor that can have a strong daily rhythm, and which is especially relevant for survival in arid environments. Furthermore,  $T_{\text{ambient}}$  values that overlap or exceed the TNZ can dramatically alter the relationship between activity timing and energy expenditure. Also, water loss was recently proposed as a mechanism that can modulate the foraging patterns of spiny mice, a species that shows a high degree of circadian flexibility (Levy et al., 2016). Interestingly, Tachinardi et al. (2015) recently reported that tuco-tucos (*Ctenomys aff. knighti*), which are diurnal in the field and nocturnal in the laboratory, spontaneously revert to a diurnal phenotype when placed in a respirometry set-up. The authors propose that tuco-tucos, which live in sealed underground tunnels, might be sensitive to small changes in air composition and humidity that can affect their circadian behavior. Another interesting finding, also in captive tuco-tucos (*Ctenomys talarum*) is that diet quality can change the daily temporal organization of behavior in this species. Comparing the distribution of *ad libitum* grass intake over the day, they found that animals maintained on a hard-to-digest grass of low energetic quality (*Cortaderia selloana*) predominantly fed during daytime, whereas the bulk intake of easy-to-digest grass (*Bromus unioloides*) occurred during the dark hours (Martino et al., 2007). Also of note, a recent study compared the daily distribution of activity in kaluta (*Dasykaluta rosamondae*), a small Australian marsupial, which is diurnal during the colder winter (proposed to save energy) and nocturnal during the warm summer (proposed to avoid predation and dehydration), indicating that factors influencing daily temporal niche selection can be subject to seasonal change (Pavey et al., 2016). Thus, food abundance, food quality, water balance, thermoregulation, energy homeostasis and predation might all be determinants of the circadian phenotype.

## Conclusions

Here, we have reviewed the WFF paradigm as a method to explore the flexibility of daily rhythms in mice. For small endothermic animals, such as many rodents, thermoregulatory costs can consume a significant portion of the daily energy budget. Our findings that negative energy balance (i.e. a change in the homeostatic balance) induces a diurnal phenotype illustrates that daily rhythms can be shaped and flexibly aligned to the light–dark environment in a way that would improve fitness in nature. Endothermic burrowing mammals can reduce their daily thermo-energetic costs significantly by becoming diurnal, as they avoid the coldest ambient temperatures at night, thus encountering higher average temperatures throughout their circadian cycle. Also, non-burrowing animals, when exposed to  $T_{\text{surface}}$  rhythms that remain below thermo-neutrality, gain an energetic benefit from diurnality when they employ heat-conserving behavioral and physiological mechanisms during the cold night (Fig. 2). A shift towards a diurnal phase might come at the cost of increased predation risk, and the optimal phasing of circadian rest–activity cycles in a natural environment thus involves the integration of multiple trade-offs, rather than being set by single timing cues (like light) alone (Fig. 3). The undisturbed light entrainment of the SCN will remain a reliable predictor of the thermal environment, which is especially important in burrowing animals that do not have continuous information on surface light and temperature. The SCN, as an internal representation of the external light–dark cycle, thus plays a critical role in optimizing activity patterns, such

that predation risks are balanced against thermo-regulatory costs. For humans, understanding the flexibility in the timing of output rhythms might reduce the negative consequences of non-conventional lifestyles (like shift work) or help athletes to optimize their physical performance to the time of an event. Also, the intimate link between metabolic balance and circadian rhythms could suggest interesting new approaches to the prevention and treatment of obesity or metabolic problems. In general, we also hope that our data indicate that habitat and context (of humans and animals alike) actively regulate our functioning; a factor that is frequently underestimated or ignored. Understanding the diversity of responses in complex natural situations can, in some cases, provide novel insights that are easily missed in controlled experiments. Thus, bringing the fields of ecology, physiology and neuroscience closer together would be of interest to all parties involved.

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## Competing interests

The authors declare no competing or financial interests.

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