Chapter 10

Synthesis

Vincent van der Vinne
The circadian system structures the timing of physiology and behavior of organisms and thereby allows the optimization of internal processes to the daily changes in the environment. Temporal specialization to a specific temporal niche optimizes the internal organization of organisms to the rhythmic outside world but simultaneously constrains activity in other temporal niches (Kronfeld-Schor and Dayan, 2003). Experiments measuring daily activity rhythms under natural conditions show substantial differences in the temporal organization of small mammals compared to the laboratory. While mice are strictly nocturnal under standard laboratory conditions, outside conditions can result in diurnal activity rhythms (Daan et al., 2011). This thesis explored causes and consequences of such plasticity in the daily timing of activity.

Environmental factors driving temporal niche switching

Studies reporting daily activity rhythms of small mammals under natural conditions show distinct differences from the daily rhythms observed in the laboratory (Fenn and Macdonald, 1995; Begall et al., 2002; Levy et al., 2007; Gattermann et al., 2008; Daan et al., 2011; Tomotani et al., 2012). Plasticity in the daily timing of activity has been linked to environmental changes in food availability (Daan et al., 2011), ambient temperature (Fulk, 1976), interspecies competition (Shkolnik, 1971), season (Hoogenboom et al., 1984) and predation risk (Fenn and Macdonald, 1995). Overall, examples of temporal niche switching (reviewed in Chapter 2) show the plasticity of the circadian system in response to environmental changes.

This thesis focusses on the influence of energetic challenges on the daily distribution of activity. The circadian thermo-energetics (CTE) hypothesis predicts that diurnality is associated with energy savings and that typically nocturnal endotherms therefore become diurnal in response to reduced food intake or elevated energy expenditure (Chapter 2). In line with this prediction, the intensity of food anticipatory activity (FAA) in response to time-restricted feeding is increased when food intake is reduced (Mendoza et al., 2005b; Gallardo et al., 2014). Reducing food intake without timed feeding also makes nocturnal mice active at daytime (Hut et al., 2011) and this effect is enhanced when energy expenditure is elevated by low ambient temperatures (Chapter 3) or lactation (Perrigo, 1987). The importance of energetic challenges in shaping the daily distribution of activity was further highlighted by the ‘working for chocolate’ protocol, which did not reduce energy availability, and—presumably therefore—failed to increase daytime activity (Chapter 4). The notion that negative energy balance or energetic challenge in general would stimulate diurnal activity in otherwise nocturnal mice under laboratory conditions, was further substantiated under more natural conditions. The food availability manipulations performed in our outdoor enclosures indeed showed that the amount of daytime activity of mice living under semi-natural conditions depended on energy availability (Chapter 8).
The risk of overheating in response to high ambient temperatures presents another kind of energetic challenge to endotherms (Scholander et al., 1950; Gordon, 2012). Reducing activity during the warmest part of the day allows animals to reduce heat dissipation difficulties. Indeed, exposure to high ambient temperatures or solar radiation reduces activity levels during the middle of the light phase (Fulk, 1976; Bacigalupe et al., 2003; Váčzi et al., 2006). The shifted daily distribution of activity and nest attendance observed in lactating common voles housed in constant ambient temperatures (Chapter 5) showed that such changes are not only a direct effect of the encountered daily temperature cycle, but indicates that the light-dark (LD) cycle is used as a proxy by the animals to anticipate warm or cold temperatures.

Day-night differences in perceived predation risk are another environmental factor which is likely to influence the daily activity rhythm (Chapter 8). Since most predators are specialized for a specific temporal niche, prey species can alter predation risk by changing their temporal niche (Daan, 1981; Gerkema et al., 2013; Chapter 7). The observation of mice under semi-natural conditions becoming strictly nocturnal in response to the absence of covered runways suggests that the perceived predation risk shapes the daily distribution of activity of mice (Chapter 8). Our results are in line with previous studies showing that high (perceived) predation risks during the night (Fenn and Macdonald, 1995; Bakker et al., 2005) or day (Kitchen et al., 2000) drive activity rhythms of the prey species toward the opposite temporal niche. The transient changes in the daily activity rhythm following runway cover manipulations showed that the observed changes in activity timing are not a direct masking effect but suggest regulation by an internal oscillatory system involved in the regulation of daily activity patterns.

The shift to diurnality induced by energetic challenges in the ‘working for food’ (WFF) protocol in ambient temperature cycles in-phase and in anti-phase with the LD-cycle shows that daily activity rhythms remain entrained to the LD-cycle in energetically challenging conditions (Chapter 3). The LD-cycle is thus used as a proximate cue to ultimately reduce daily energy expenditure by synchronizing the activity rhythm to the daily ambient temperature cycle. Since the LD-cycle is a better predictor of the daily ambient temperature cycle than ambient temperature cycles encountered on previous days (Chapter 6), adaptive benefits can be derived by not assessing the ambient temperature rhythm on a daily basis. Whether other environmental rhythms (e.g. predation risk rhythm) are also purely assessed on the basis of the LD-cycle remains to be studied. Daily predation risk rhythms may show less day-to-day variability and might thus provide a more reliable predictor of future daily predation risk rhythms.

Sex differences in temporal niche switching in response to changes in cover availability (Chapter 8) and WFF workload (SJ Riede, V van der Vinne, RA Hut; unpublished) indicate greater plasticity of the circadian system of males. While both sexes are capable of
shifting the daily activity rhythm, males more readily become diurnal in response to changes in the environment. Future studies should therefore focus on a sexual dimorphism in the risk taking behavior and plasticity of the circadian system. Based on our results, a positive correlation between risk taking behavior and circadian plasticity would be expected when the two sexes will be compared.

Overall, the experiments presented in this thesis demonstrate the environment’s influence on the daily timing of activity and rest. The energetic state and perceived predation risk of mice modulate daily activity rhythms. The controlled conditions used to assess the influence of energetic challenges could also be employed to assess the influence of other environmental factors on the daily timing of activity. Ultimately the interaction between conflicting environmental influences should be assessed to gain a full understanding of how animals interpret and adapt to the time structure of their environment.

Benefits of temporal niche switching

Since circadian rhythms are present in the physiology and behavior of most species, it is assumed that these rhythms provide adaptive advantages. Possible fitness benefits of a circadian organization can be divided into intrinsic and extrinsic benefits (Sharma, 2003). Intrinsic benefits can result from distributing conflicting metabolic processes to different times of the rest activity cycle (Pittendrigh, 1993) while extrinsic benefits can be derived from optimizing the daily timing of physiology and behavior to the time structure of the environment (Daan, 1981). Endogenous circadian rhythms provide extrinsic benefits by allowing organisms to anticipate daily environmental changes in light intensity, ambient temperature, predation risk, and food availability. The ultimately optimal daily distribution of activity and rest will maximize fitness by balancing the consequences of all daily environmental rhythms. Temporal niche switching allows animals to cope with changes in the environment and optimize the daily activity rhythm to the encountered conditions.

Circadian thermo-energetics hypothesis

The CTE hypothesis introduced in Chapter 2 proposes diurnality as a strategy to reduce the daily energy expenditure of endotherms living in temperate climates. Shifting the timing of activity to the warmest part of the day synchronizes the rest phase with the colder night. Since most energy saving strategies are associated with the resting phase (e.g. nest insulation, huddling, daily torpor) and these strategies provide the greatest energetic benefits when ambient temperatures are lower, diurnality is expected to reduce daily energy expenditure.

The energetic consequences of temporal niche switching were assessed in a fully quantitative model in Chapter 6. This model combined laboratory measurements of energy expenditure during the active and rest phase with the quantification of the energetic
consequences of different energy saving strategies in mice. The modeling approach used to assess the energetic consequences of diurnality allowed the identification of the temporal niche influence from other energy saving strategies used by energetically challenged animals. The systematic assessment of the factors determining daily energy expenditure revealed the importance of buffered nest temperatures in making diurnality energetically beneficial. Overall, the quantitative modeling assessment of energetic consequences of diurnality identified diurnality as an energy saving strategy for endotherms that are able to rest in sheltered locations with some degree of thermal insulation.

Shifting to diurnality is one of a number of energy saving strategies used by energetically challenged mice. When exposed to increasing energetic challenges, mice use a hierarchy of energy saving strategies to reduce daily energy expenditure while presumably minimizing other costs associated with these energy saving strategies. Increased energetic challenges can be countered by huddling with nestmates (Chapter 6, 8) and resting in insulated nests (Chapter 6, 8). Shifting to diurnal activity subsequently allows for a lowering of body temperature during the rest phase and eventually the occurrence of daily torpor at night (Chapter 3; Hut et al., 2011). The observation that males become more readily diurnal in response to energetic challenges in the WFF protocol compared to females (SJ Riede, V van der Vinne, RA Hut; unpublished) suggests a sex difference in the hierarchy of energy saving strategies.

The energetic benefits of diurnality will ultimately be balanced against other consequences of the daily activity rhythm. Chapter 7 determines the optimal temporal niche quantitatively when the encountered energetic benefits of diurnality, foraging yield and predation risk rhythms are all taken into account. Although this analysis did not incorporate reproductive success, it illustrated the importance of assessing different consequences of temporal niche switching simultaneously. Ultimately, the optimal temporal distribution of activity and rest depends on the environment encountered by an animal. Whether animals are capable of adapting their daily activity rhythm to the interaction of environmental factors determining the optimal temporal organization remains to be tested.

The CTE hypothesis predicts that diurnal endotherms will not phase shift in response to reduced energy availability (Chapter 3). High energy intake can however be compensated by re-organizing the daily timing of activity and rest (Chapter 5). In our modern-day society, the abundance of food might thus result in a wider range of human chronotypes by allowing some people to develop a later chronotype where much of the active phase extends in the night. Since suprachiasmatic nucleus (SCN) phase seems insensitive to changes in metabolic state (Buhr et al., 2010; Chapter 3), the modulation of chronotype by metabolism could be studied by assessing the discrepancy between SCN-driven melatonin rhythms and the daily behavioral rhythm.
Mechanisms responsible for circadian plasticity

The circadian system, consisting of circadian oscillators throughout the body orchestrated by the SCN (Dibner et al., 2010), is entrained to the environmental LD-cycle in conditions of normal energy availability (Pittendrigh and Daan, 1976). Under WFF conditions, the mice eventually become diurnal by advancing their phase angle of entrainment: they start running in their running wheels earlier every day when the workload is increased. It was essential to test whether the mice were selectively choosing to become active during the daytime, as predicted by the CTE hypothesis, or whether the increased need to eat at the end of the rest phase was causing this early rise. When hunger is causing the advancing activity and thereby ignoring the LD-cycle, mice would continue to advance their activity onset, even when the LD-cycle was phase delayed. The Chapter 3 shows that mice delayed there active phase when the LD-cycle was delayed and advanced when the LD-cycle was advanced. This shows that mice were selectively choosing to be active during the light phase. The transient re-entrainment of activity in response to phase advances and delays of the LD-cycle (Chapter 3) shows that an endogenous circadian rhythm maintain entrainment to the LD-cycle under the energetically challenging conditions of the WFF protocol.

Circadian re-organization: SCN versus liver and adrenal

The phase distribution between oscillators in different organs is altered by metabolic challenges (Chapter 3). While the SCN remains phase-locked to the LD-cycle, peripheral oscillators in the liver and adrenals are phase advanced. Challenging nocturnal mice energetically, results in a reorganization of oscillators that resembles that of diurnal mammals (Lambert and Weaver, 2006). The role of the SCN as orchestrator of peripheral oscillators and physiological rhythms has been extensively studied under ad libitum feeding conditions (e.g. Kalsbeek et al., 2011). The metabolic changes responsible for the phase advance of physiology and behavior was however not associated with a phase shift in PER2 clock gene (Chapter 3) or immediate early gene FOS (M Verreij, SJ Riede, V van der Vinne, RA Hut; unpublished) expression in the SCN. This observation is in line with earlier reports that SCN phase does not change in response to changes in corticosterone (Oster et al., 2006) and body temperature rhythms (Buhr et al., 2010), while the phase of peripheral oscillators does. These experiments identify the SCN as an internal representation of the external LD-cycle, which provides the body with a reliable internal clock unaffected by endogenous and environmental changes.

The observation that activity remains entrained to the LD-cycle and not the ambient temperature cycle during WFF (Chapter 3) indicates that the SCN retains a central role in the regulation of daily activity rhythms in energetically challenged mammals. The role of
the SCN in regulating seasonality in response to changes in photoperiod provides a functional explanation for the unresponsiveness of the SCN to endogenous changes. The circannual pacemaker driving seasonal changes in reproductive state uses the SCN-controlled melatonin rhythm as a measure for day length (Hut, 2011; Chapter 9). Fluctuations in the phase relationship between the LD-cycle and SCN would make the melatonin signal an unreliable readout of the external photoperiod. Proper annual timing thus requires a SCN which phase only depends on the external LD-cycle and not on endogenous changes driven by metabolism and/or stress.

Peripheral oscillators in the liver and adrenals are, unlike the SCN, phase advanced in response to energetic challenges (Chapter 3). Similar phase shifts have been reported in peripheral tissues in response to time-restricted daytime feeding (Damiola et al., 2000; Stokkan et al., 2001). These peripheral phase shifts adjust physiology of these organs (Kornmann et al., 2007) to be in line with the diurnal activity rhythm. Phase synchrony within and between peripheral oscillators is maintained through a combination of daily rhythms in feeding (Damiola et al., 2000), corticosterone (Le Minh et al., 2001; Pezük et al., 2012) and neural communication from the SCN (Guo et al., 2005; 2006; Vujovic et al., 2008). Since the SCN was not phase shifted and adrenalectomy did not result in behavioral changes in the WFF-induced shift to diurnality (V van der Vinne, SJ Riede, RA Hut; unpublished), the altered feeding rhythm is a likely candidate to be responsible for the phase advance of peripheral oscillators.

The diurnal activity pattern of energetically challenged mice was also associated with a shifted corticosterone rhythm, peaking during the middle of the light phase (Chapter 3). Daily corticosterone rhythms are regulated by a combination of autonomous nervous system input, pituitary hormones and the adrenal clock (Oster et al., 2006; Kalsbeek et al., 2011). Since the SCN (Chapter 3) and pituitary rhythms (V van der Vinne, MT Sellix, M Menaker, RA Hut; unpublished) were not phase shifted by the WFF protocol, the shifted corticosterone rhythm is likely a result of the altered rhythmicity of the adrenal clock. Since corticosterone release is gated by the adrenal clock (Oster et al., 2006), a phase advance of this adrenal clock could make the adrenal more sensitive to ACTH stimulation during the day, in addition to corticosterone release driven by the local shifted adrenal clock.

Mechanisms underlying diurnality: relationship with the food entrainable oscillator

This thesis presents experiments in which mice were energetically challenged without restricting the access to food to a specific time of day (Chapter 3, 8). These experiments show that mice respond to energetic challenges by becoming diurnal but the mechanisms responsible for such an endogenously driven phase advance are unknown.

The anatomical substrate resulting in daytime activity has previously been investigated using time-restricted feeding protocols (reviewed in Davidson, 2009; Mistlberger, 2011).
Time-restricting food access to a few hours during the light phase results in FAA in the hours preceding food availability (Mistlberger, 1994; Stephan, 2002). FAA persists during periods of total food deprivation, entrains transiently to changed mealtimes and persists following SCN ablation (Stephan et al., 1979). These findings show that FAA is driven by a SCN-independent ‘food entrainable oscillator’ (FEO; Mistlberger, 1994; Stephan, 2002). A large number of lesion studies have been unsuccessful in localizing the FEO (reviewed in Davidson, 2009), resulting in the hypothesis that the FEO is comprised of a dispersed network of oscillators (Acosta-Galvan et al., 2011).

Figure 10.1. Regulation of daytime activity by time-restricted feeding (A) and working for food (B).

The time-restricted feeding protocols used to induce FAA provide both an energetic and a timing signal which might each result in increased daytime activity (Challet and Mendoza, 2010). This thesis showed that energetic challenges (Chapter 3) and timed feeding (Chapter 4) are both separately sufficient to induce diurnality in mice. If energetically- and timed-feeding induced diurnality are driven by distinct neural networks (Webb et al., 2009; Blum et al., 2012), FAA likely results from an interaction between these regulatory networks (Fig. 10.1). Identifying the neural substrate of the FEO would thus require the identification of the neural substrates responsible for the energetically- and timed-feeding induced daytime activity. Since these regulatory networks are complementary and separately capable of inducing diurnality, modulations in one network will be compensated by the other network when assessed using time-restricted feeding protocols.
The mechanisms responsible for the endogenously driven shift to diurnality in response to energetic challenges can be studied using the WFF protocol. The lack of a phase shift of the SCN clock-gene rhythm (Chapter 3) indicated that mechanisms responsible for the shift to diurnality must be downstream or parallel to the SCN. The transient re-entrainment patterns following LD-cycle shifts (Chapter 3) showed that the diurnal activity patterns are driven by a light-entrainable oscillator. These observations could result from different regulatory mechanisms (Fig. 10.2).

Figure 10.2. Possible regulatory mechanisms driving temporal niche switching in response to energetic challenges. See text for details.

The shift to diurnality observed in response to energetic challenges might result from a mechanism whereby the period of the activity rhythm is dictated by the SCN but the preferred SCN phase to be active depends on the energetic state (Fig. 10.2A). The timing of behavior might also be controlled by both the SCN and an ‘activity oscillator’ positioned
parallel or downstream to the SCN. A parallel oscillator model requires direct (non-SCN) LD-input while metabolic feedback would control the relative influence of the SCN and ‘activity oscillator’ on the activity rhythm (Fig. 10.2B). An ‘activity oscillator’ located downstream from the SCN would require timing input from the SCN to entrain to the LD-cycle. The coupling strength of the SCN on the ‘activity oscillator’ would be determined by metabolic feedback (Fig. 10.2C).

![Figure 10.3. Working for food (WFF) in constant light conditions (LL) results in two behavioral periods in the activity rhythm. Two representative actograms of mice housed in constant light undergoing WFF (starting on day 0) are shown. Prolonged exposure (>3 months) to LL resulted in a single behavioral period (~25 h; top of actograms). WFF resulted in two behavioral period as shown in the periodogram analyses (day 14-75). (V van der Vinne, SJ Riede, RA Hut, unpublished)](image)

Discerning between these different regulatory mechanisms requires a set of experiments. Activity rhythms of mice undergoing WFF in constant light conditions (LL) support the hypothesis of an ‘activity oscillator’ (Fig. 10.3). Exposure to LL in mice typically lengthens the circadian period and prolonged exposure to LL weakens the output of the SCN and eventually could lead to arhythmicity (Spoelstra and Daan, 2007). CBA/CaJ mice
under LL conditions (>3 months) and ad libitum feeding conditions did not lose circadian rhythmicity in behavior, but did show the expected lengthening of the circadian activity rhythm (~28h) in all mice. Weakening the output of the SCN under LL might reveal the nature of the circadian activity oscillator, if it exists (Fig. 10.2A vs. Fig. 10.2B/C). When mice under LL were transferred from ad libitum feeding to WFF conditions, the activity bout was split in two bouts, one with an ~24h period and one oscillating with an ~28h period (Fig. 10.3). The presence of two behavioral periods in the activity pattern of these energetically challenged mice showed the presence of an ‘activity oscillator’, which is sensitive to energetic challenges.

The experiment presented in Fig. 10.3 provides preliminary proof that an additional activity oscillator may exist parallel or downstream from the SCN. Distinguishing between the parallel ‘activity oscillator’ (Fig. 10.2B) or the downstream ‘activity oscillator’ (Fig. 10.2C) can be done using SCN-lesion experiments. Performing WFF in a LD-cycle should result in entrained activity rhythms if the ‘activity oscillator’ is parallel to the SCN while a downstream ‘activity oscillator’ would not be able to entrain the activity rhythm of SCN-lesioned mice.

Human implications of plasticity in daily timing

This thesis attempts to understand the causes and consequences of plasticity in the daily timing of activity at a fundamental level, but the presented findings may also have important societal implications. For instance, understanding how SCN output shapes timing of the sleep-wake cycle and rhythms in peripheral tissues will have consequences for understanding causes and consequences of the extremely wide distribution of sleep phase in modern human society (Roenneberg et al., 2007). The distribution of human individuals’ chronotypes is increased by exposure to the modern human society (Wright et al., 2013), but the causes and consequences of altered activity timing remain largely unknown. The influence of the energetic state of an animal on the timing of activity, studied in this thesis, provides a possible explanation for the large variation in chronotypes observed in humans. A study performed during this PhD project assessed how high school performance is changes with time of day in different chronotypes (van der Vinne et al., 2015). The relationship between chronotype and age in humans suggests that the occurrence of late chronotypes is related to the development of children, leading to a peak of late chronotypes during adolescence (Roenneberg et al., 2007). High school students thus face a mismatch between their endogenous late chronotype and early school hours imposed by societal schedules. Late chronotypes obtained on average lower school grades, but this effect could be ameliorated by testing later in the day (van der Vinne et al., 2015). A better understanding of the causes resulting in variation in chronotypes (e.g. metabolic state;
Roenneberg et al., 2012) could be used to reduce the mismatch between internal time and societal schedules.

Further applications of fundamental knowledge on the regulation of circadian phase might be found in human health care. Development of personalized medicine will optimize health care and should include optimizing the timing of medication and treatment for individual patients (chronotherapy). Understanding the relationship between the behavioral sleep-wake cycle and the timing of physiology and toxicology would be essential in developing personalized treatments. Measuring individual phase and understanding how and why some people have late sleep-wake cycles while others are early will form an essential component in chronotherapy. The evolutionary approach to understand the behavioral phenomenon of temporal niche switching may therefore ultimately contribute to human health care.