Plasticity in daily timing of behavior

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Chapter 1

General introduction

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The 24h light-dark cycle on earth presents all living organisms with a daily changing environment. To optimize the timing of physiology and behavior to these daily environmental changes, organisms have evolved circadian oscillators. These endogenous circadian clocks have a period of approximately 24 hours when external time cues are absent. It is hypothesized that endogenous clocks provide organisms with the opportunity to synchronize conflicting body processes, prevent exposure to adverse environmental conditions and predict predation risk, foraging success and energy expenditure (Daan, 1981; Pittendrigh, 1993; Fenn and Macdonald, 1995; DeCoursey et al., 2000; Bakker et al., 2005).

In mammals, almost all cells contain an intracellular clock that produces a ~24h oscillation via a transcription-translation feedback loop. The core-clock loop consists of a positive limb (CLOCK, BMAL1) stimulating the expression of clock genes of the negative loop (PER, CRY) which inhibits the activity of the positive limb (reviewed in Ukai and Ueda, 2010). At the organismal level, the main clock is located in the suprachiasmatic nucleus (SCN) which is reset daily by the environmental light-dark cycle (reviewed in Welsh et al., 2010). Clocks located in other brain areas and peripheral organs receive timing information from the SCN and other zeitgebers such as feeding time (reviewed in Dibner et al., 2010). These different regulatory levels together control daily rhythms in physiology and behavior.

Most studies investigating the mechanisms of circadian rhythms monitor model organisms under laboratory conditions, but not much is known about the preferred temporal niche of these species in the wild. The few studies of small mammals' activity rhythms under natural conditions report substantial differences in daily activity rhythms in the wild compared to laboratory settings (Levy et al., 2007; Gattermann et al., 2008; Daan et al., 2011; Tomotani et al., 2012). Furthermore, long-term activity registrations of mice under natural conditions report frequent temporal niche switching, presumably in response to changing environmental conditions (Daan et al., 2011). The daily activity rhythms of small mammals subjected to outside conditions is thus much more plastic than laboratory studies suggest. This thesis explores the underlying causes and consequences of such circadian plasticity with a special focus on the role of metabolism in modulating circadian rhythms.

Circadian control of metabolism

The circadian modulation of metabolism might be a crucial component for circadian rhythms in physiology and behavior (Bass and Takahashi, 2010). Circadian oscillators in the SCN and peripheral oscillators together control the regulation of metabolism on both cellular and systemic levels (Kalsbeek et al., 2010). Disruption of circadian rhythmicity of these regulatory pathways has been shown to result in metabolic disfunction (Turek et al., 2005; Marcheva et al., 2010; Sadacca et al., 2010; Gerhart-Hines et al., 2014). In humans,

disruption of circadian rhythms has been associated with metabolic disorders, such as an elevated risk for obesity and type II diabetes (Scheer et al., 2009; Pietroiusti et al., 2010; Pan et al., 2011; Roenneberg et al., 2012).

The SCN modulates metabolism in peripheral organs and tissues through systemic factors (Balsalobre et al., 2000; Brown et al., 2002; Guo et al., 2005; Vujovic et al., 2008) including hormonal release from the pituitary and through the autonomous nervous system (Kalsbeek et al., 2011). The daily rhythm in corticosterone release is regulated by circadian oscillations in the SCN and adrenal glands. The SCN controls the release of hypothalamic releasing factors and sets the sensitivity of the adrenal for these humoral factors via the autonomic nervous system (Kalsbeek et al., 2011) while the local adrenal clock modulates the daily rhythm in corticosterone release (Oster et al., 2006). The regulation of blood glucose concentration is another metabolic process tightly regulated by the circadian system. Synergistic sympathetic and parasympathetic inputs to the liver and pancreas are modified by the SCN to control fluctuations in plasma glucose concentration over the course of the day (Kalsbeek et al., 2011). Glucose release from the liver is stimulated by sympathetic activity prior to the active phase (Cailotto et al., 2005) while insulin release is reduced during the rest phase by inhibitory SCN outputs (Kalsbeek et al., 2008a).

Further circadian control of metabolism is exerted through the regulation of gene expression in different organs (Kita et al., 2002; Storch et al., 2002; Panda et al., 2002a; Zambon et al., 2003). This local control of gene expression is believed to enable the optimal daily timing of a specific set of rhythmic genes for each organ. Circadian oscillators in peripheral organs receive time-of-day information from the SCN through direct neural and hormonal communication, feeding cues and body temperature rhythms resulting in a stable phase distribution of peripheral oscillators (Yamazaki et al., 2000; Stokkan et al., 2001; Brown et al., 2002; Buijs et al., 2003; Terazono et al., 2003; Guo et al., 2005; 2006; Vujovic et al., 2008; Buhr et al., 2010).

Metabolic feedback on circadian clocks

Experiments modulating metabolic state reveal the influence of metabolism on circadian rhythmicity. Changes in cellular metabolism have been shown to interact with the core translational-transcription loop (Bass and Takahashi, 2010). High $NAD^+/NADH$ ratios, signaling low intracellular energy levels, can modulate the core clock network in multiple ways (Rutter et al., 2001; Asher et al., 2008; Nakahata et al., 2008), resulting in altered clock gene expression (Asher and Schibler, 2011).

A metabolically induced change in the circadian organization of behavior can be observed in the food anticipatory activity (FAA) induced by time-restricted feeding in rodents (Mistlberger, 1994; Stephan, 2002). FAA transiently re-entrains to changing mealtimes, persists during complete food deprivation and following SCN ablation (Stephan et al.,

1979), showing that FAA is driven by an SCN-independent 'food entrainable oscillator' (FEO). Lesion studies aiming to identify the anatomical location of the FEO have been unsuccessful (reviewed in Davidson, 2009) leading to the hypothesis that the FEO consists of a dispersed network of oscillators (Davidson, 2009; Mistlberger, 2011).

A number of experiments indicate that these different oscillators driving FAA are phase shifted by different signals, namely the timing of food and the energetic challenge resulting from reduced food intake, which is a crucial component of food restriction experiments inducing FAA (Challet and Mendoza, 2010). The importance of food timing in modulating the daily distribution of activity and rest has been demonstrated in experiments where *ad libitum* fed rodents received a timed palatable meal. Timing a palatable chocolate meal during the light phase to animals with *ad libitum* access to regular chow can induce FAA (Mistlberger and Rusak, 1987; Mendoza et al., 2005a; Verwey et al., 2007; Angeles-Castellanos et al., 2008; Hsu et al., 2010) and this is associated with shifted gene expression in brain regions involved in the reward system (Mendoza et al., 2005a; Angeles-Castellanos et al., 2007; Verwey et al., 2007; Waddington Lamont et al., 2007; Angeles-Castellanos et al., 2008). The intensity of FAA induced by timed palatable food access is however much lower than that observed following complete food restriction, showing that the timing of food is not the only factor driving FAA.

The energetic challenge presented by reducing food intake is another factor modulating the daily distribution of activity. Inducing FAA is typically accomplished by restricting food intake to 60-80% of *ad libitum* food intake and such reductions in food intake result in more pronounced FAA . To identify the effect of reduced food intake *per se*, we designed the 'working for food' (WFF) protocol. The WFF protocol reduces food intake by letting mice earn food at their own preferred time of day by running in a wheel. Increasing the workload reliably results in a diurnal activity pattern (Hut et al., 2011). Together, these experiments implicate energetic state of the animal as a modulating factor of the daily distribution of activity. This thesis aims to experimentally modulate the energetic state of mice to assess its influence on the daily timing of behavior.

Thesis overview

One of the main topics of this thesis is how the daily activity rhythm is shaped by environmental influences. The first chapter (**Chapter 2**) presents a review of existing literature for examples of temporal niche switching between and within species. The main focus of this review is the identification of environmental factors driving temporal niche switching. Based on these reported modulating factors, we proposed the circadian thermo-energetics (CTE) hypothesis as a theoretical framework for understanding temporal niche switching in energetically challenged small mammals.
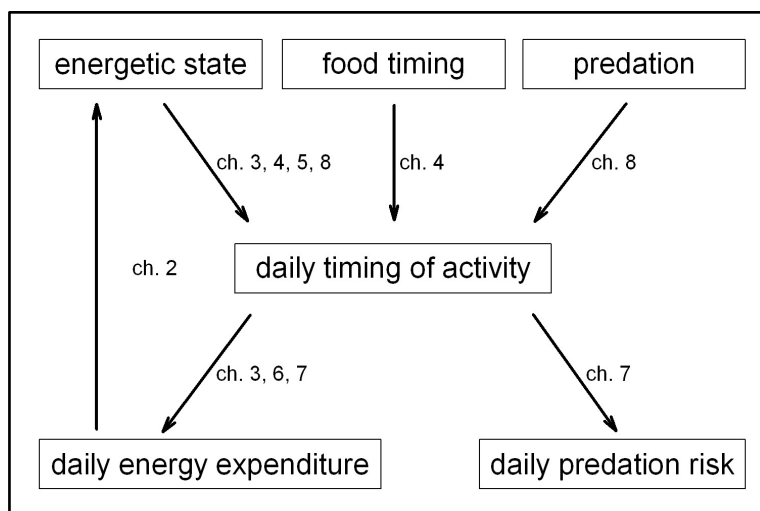
Figure 1.1 Thesis overview. The influence of different environmental factors on the daily timing of activity are assessed as well as the consequences of changes in the daily timing of activity.

The isolated influence of energetic challenges on the daily distribution of activity is assessed in a number of laboratory experiments. The influence of energetic state on the daily distribution of activity of mice is tested experimentally in **Chapter 3** by a combination of experiments in which energy intake is reduced and energy expenditure is increased. The importance of energetic challenges in modulating daily activity rhythms is further assessed in the 'working for chocolate' protocol which allows mice to earn a food reward without being energetically challenged (**Chapter 4**). Both of these chapters highlight how small mammals change their daily activity rhythm when energy becomes scarce. Conditions of easy energy availability were animals attempt to maximize their energetic turnover are assessed in **Chapter 5**. Here the behavioral adaptations to high ambient temperatures are measured in lactating common voles.

The modulation of activity rhythms by energetic challenges is further assessed under semi-natural conditions in **Chapter 8**. Mice living in outdoor enclosures exposed to natural fluctuations in light and temperature undergo manipulations of food availability to simulate conditions of food shortage. The influence of another environmental factor, namely the effect of changes in perceived predation risk, on daily activity rhythms is also studied in outdoor enclosures. The final chapter of this thesis deals with the regulation of seasonal rhythms (**Chapter 9**). The influence of photoperiod and the plant metabolite 6-MBOA, whose abundance peaks in spring, on the internal seasonal switch is measured in female common voles.

The mechanisms underlying plasticity in daily rhythms are explored in chapters 2, 3 and 4. **Chapter 2** presents a number of theoretical mechanisms which might underly temporal niche switching and reviews morphological constraints limiting plasticity of activity rhythms. The mechanisms responsible for temporal niche switching in response to energetic challenges specifically are explored in a number of experiments in **Chapter 3**. First, the continued importance of the light-dark cycle for the entrainment of daily activity rhythms is shown in energetically challenged mice undergoing the WFF protocol. Second, the phase distribution of circadian clocks throughout the body is mapped, revealing a circadian re-organization of clocks in response to energetic challenges. Finally, the roles of the reward system and brain regions involved in metabolic regulation in shaping the daily distribution of activity are separated in **Chapter 4**.

The consequences of temporal niche switching for daily energy expenditure and predation risk are assessed quantitatively in three chapters. The prediction of the CTE hypothesis that diurnality lowers energy expenditure is assessed in **Chapter 3** using a model calculating daily energy expenditure based on the difference between outside and nest temperature rhythms. **Chapter 6** extends this approach by presenting a fully quantitative model of the daily energy expenditure of mice under natural conditions. A combination of energetic measurements under laboratory conditions with outdoor measurements of natural nest insulation and outside chill is combined with daily temperature cycle information to calculate the daily energy expenditure of mice. This model is used to assess the energetic consequences of temporal niche switching and how these depend on the environment an animal is encountering. Finally, the model is used to predict the energetic benefits of diurnality in different European locations and on separate days to assess the influence of day-to-day changes in temperature cycles.

The quantification of daily energy expenditure is then used to assess the optimal temporal niche for different combinations of foraging yield and day-night differences in predation risk (**Chapter 7**). Assuming that the animal is in metabolic balance, the active phase length of nocturnal and diurnal mice can be calculated for all possible foraging yields encountered under natural conditions. Reductions in active phase length enabled by diurnality have to be balanced against the changes in predation risk associated with this different temporal niche. The ultimate optimal temporal niche is therefore determined by calculating the daily predation risk of nocturnal and diurnal mice facing all possible combinations of foraging yield and day-night differences in predation risk.

Finally, the synthesis chapter (**Chapter 10**) describes, based on experiments presented in this thesis, how plasticity in the daily timing of activity is driven by environmental factors and provides adaptive benefits to mammals subjected to changing environmental

conditions. The relevance of our findings for understanding the regulation of FAA by the FEO are discussed and possible mechanisms responsible for plasticity in the daily timing of activity are presented. Finally, a number of implications for humans of the studies presented here are discussed.