Behavioral responses to combinations of timed light, food availability, and ultradian rhythms in the common vole (*Microtus arvalis*)

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Abbreviations:

CG: Centre of Gravity, FA: food Availability, LD: Light-Dark, LP: Light Pulse, SCN: Suprachiasmatic Nuclei, T: Period of external Zeitgeber cycle; LEO: Light Entrainable Oscillator, FEO: Food Entrainable Oscillator

ABSTRACT

Light is the main entraining signal of the central circadian clock, which drives circadian organization of activity. When food is made available during only certain parts of the day, it can entrain the clock in the liver without changing the phase of the central circadian clock. While a hallmark of food entrainment is a behavioral anticipation of food availability, the extent of behavioral alterations in response to food availability has not been fully characterized. We have investigated interactions between light and temporal food availability in the timing of activity in the common vole. Temporally restricted food availability enhanced, or attenuated, re-entrainment to a phase advance in light entrainment when it was shifted together with the light or remained at the same time of day, respectively. When light entrained behavior was challenged with temporal food availability cycles with a different period, two distinct activity components were observed. More so, our data indicate that in the presence of cycles of different period length of food and light, an activity component emerged that appeared to be driven by a free-running (light-entrainable) clock. Because we have previously shown that in the common vole altering activity through running-wheel availability can alter the effectiveness of food availability to entrain the clock in the liver, we included running-wheel availability as a parameter that alters the circadian/ultradian balance in activity. In our protocols, running-wheel availability enhanced the entraining potential of both light and food availability in a differential way. The data presented here show that in the vole activity is a complex of individually driven components and that this activity is, itself, an important modulator of the effectiveness of entraining signals such as light and food. (Author correspondence: vanderveen@nd.edu)

Keywords: Entrainment, Food restriction, Light, Zeitgeber, Running wheel, Ultradian

INTRODUCTION

Overt circadian rhythmicity in behavior has been shown to be driven by the clock in the suprachiasmatic nuclei (SCN) of the hypothalamus. After lesioning of the SCN, circadian rhythmicity of behavior and physiology disappears (Moore & Eichler, 1972; Stephan & Zucker, 1972), but it can be restored by transplantation of (fetal) SCN tissue (Ralph et al., 1990). The major Zeitgeber for the SCN is light, and the SCN is regarded as being part of the Light Entrainable Oscillator (LEO). Animals can also entrain their behavior to cycles in food availability (Castillo et al., 2004; Mistlberger, 1993; Stephan, 1986). Stephan (1979) showed that this also holds true for SCN-lesioned rats, and since then food availability has gained interest as a Zeitgeber for a self-sustained oscillator system outside the SCN.

Temporal food availability has thus been shown to be an alternative Zeitgeber. Krieger *et al.* (1977) reported circadian rhythms in plasma corticosteroid levels and body temperature in rats that were entrained by a regime of 2 h of food/day. Timing of feeding (itself normally in phase with other behaviors that are under the control of the SCN) synchronizes the liver's circadian clock, without altering phase of the LEO in the SCN (Damiola et al., 2000; Stokkan et al., 2001). Typically, when an animal is subjected to feeding schedules, a rise in locomotor activity is seen prior to the restoration of the food availability, and this food anticipatory activity is one of the main arguments for an oscillator underlying food entrainment (the Food Entrainable Oscillator [FEO], for review see Mistlberger, 2009; Stephan, 2002).

We have previously shown that in the common vole (*Microtus arvalis*), the molecular clock in the liver does not exhibit circadian oscillations of clock genes under *ad libitum* feeding conditions, but that these can be induced by restricting food availability to a certain part of the day and/or providing a running wheel (Van der Veen et al., 2006). The vole is an ultradian forager, feeding every 2-3 h (Daan & Slopeema, 1978; Gerkema & van der Leest, 1991), but by making a running wheel available to these voles, a circadian activity component is emphasized (Gerkema et al., 1990; Van der

Veen et al., 2006). Using hypothalamic lesions, it has been shown that it is not the SCN, but the retrochiasmatic area of the hypothalamus which is needed for the generation of these ultradian behavioral rhythms (Gerkema et al., 1990). The rather weak circadian system in voles, even resulting in a loss of circadian rhythmicity in constant dim light, has been shown to be accompanied by low amplitudes of transcriptional activity of clock-related genes of the SCN (Van der Veen et al., 2008). The attenuating effect of an ultradian behavioral rhythm on the food-entrainable clock in the liver, which occurred without a running wheel and under *ad libitum* feeding conditions, raises questions about the nature of the interaction of activity and food and light entrainment in the vole.

Effects of activity on timing of circadian activity have been shown under different conditions. Rodents have been entrained to daily activity bouts elicited by cage cleaning (Mrosovsky, 1988) or forced running (Marchant & Mistlberger, 1996; Mistlberger, 1991) as well as voluntary running (Edgar & Dement, 1991) in a wheel. In hamsters, re-entrainment to a phase advance in light is sped up by inducing running-wheel activity prior to phase advancing the LD cycle (Mrosovsky & Salmon, 1987), and such timing of activity can also change phase of entrainment (Mistlberger & Holmes, 2000). In hamsters, the ability to be active is essential for behavioral phase shifting in response to a dark pulse (Reebs et al., 1989). These reports indicate that activity can modulate photic entrainment of behavior.

Using the vole to study the three-way interaction of light, food, and activity in driving of activity can give us new insights, because the vole switches between ultradian and circadian activity patterns, and this activity, itself, has been shown to affect food as an entraining signal for the liver clock. We used a single light pulse to entrain voles, minimizing negative masking of activity (Aschoff, 1960; Mrosovsky, 1999), and we challenged the light entrainment both by food restriction and changes in the ultradian/circadian behavioral patterns through running-wheel availability.

METHODS

Animals

Common voles (*Microtus arvalis*) were bred in-house in a lab-based colony which was founded by individuals trapped in the Netherlands (53°20'N:6°16'E) and subsequently bred in-house for multiple generations. Male common voles were housed individually in translucent cages within visual and audible range of each other, with the provision that adjacent cages belonged to different experimental groups. Cages were placed in a sound-attenuated, climate controlled ($22 \pm 1^\circ\text{C}$; 70% relative humidity) room, and all voles were initially entrained to 12 h of dark alternating with 12 h of light (LD 12:12; L = 250-350 lux, depending on cage placement, D = dim red light). After the animals were fully entrained, the 12:12 LD cycle was replaced with a single, 30-min light pulse (LP) at the beginning of the subjective night. Entrainment to a single LP was chosen to minimize negative masking of activity (Aschoff, 1960; Mrosovsky, 1999) that could interfere with activity patterns induced by running wheels and temporal food availability (FA). The LP was chosen such that it would lead to stable entrainment of the LEO by phase delays (DeCoursey, 1972; Pittendrigh & Daan, 1976). Food and light conditions and running-wheel availability varied with the experimental protocols. All experiments were approved by the Animal Experimentation Committee of the University of Groningen (DEC 2809 & 2809-1) and were performed in agreement with international ethical standards and with the standards of the journal (Portaluppi et al., 2010).

Re-entrainment to a phase advance in light

Experiment 1 was designed to assess the effect of FA timing on a phase advance of 4 h in the light entrainment (jet-lag). An additional level of variation was introduced by performing the experiment both on animals housed with and without a running wheel. After initial entrainment in LD 12:12, animals were entrained to a 30-min LP at the beginning of the subjective night for 10 days, after

which the LP was phase-advanced by 4 h. The effect of FA on entrainment was investigated by removing FA from two groups of animals during the 8 h following the LP (N = 7 per group). After the phase advance in the LP, the time of restriction either remained at the same clock time (stable FA; N=14), or was phase advanced (shifting FA; (N=14) with the LP. Common voles have not been reported to hoard food in nature, or while housed in our laboratory, but they do spill food on the cage bottom while feeding; therefore in the control group (N = 14), where food was available *ad libitum*, animals did receive a clean cage daily. Because in the absence of a running wheel, the circadian component of activity was anticipated to be weak in some cases, six voles were housed with and eight voles without a running wheel. From the voles without a running wheel, three voles in each of the groups with FA and two voles in the control group exhibited a circadian activity component that was too weak to be included in a comparative analysis. The eventual group sizes were thus six animals per group, with the exception of N = 5 for the two FA groups housed without a running wheel.

Food restriction was limited to 8 h absence of food. A period of 8 h would on average comprise three ultradian feeding bouts, and previous work indicated that restricting food for longer than 8 h leads to weight loss (Van der Veen et al., 2006), which would add an unwanted caloric food restriction to the paradigm. Cage bedding was refreshed every day preceding the food restriction, also for the animals that had *ad libitum* FA.

Activity components related to light, food, and running-wheel availability

Behavioral phasing in the presence of timing cues

Experiment 2 was designed to investigate the contributions of the ultradian activity component and the light and FA-entrained components to overall activity. Animals were again entrained to the 30 min LP every 24 h for 10 days. After these 10 days, the cycle length (T) of the LP was reduced from T

= 24 to $T = 23.5$ h. After a period of 10 cycles of the LP with $T = 23.5$ h, animals were introduced to a FA-cycle of 8 h during which FA was restricted and 17 h of FA ($T = 25$). Two groups of animals were either given the first episode of 8 h of food restriction starting directly after lights off ($N = 16$), i.e., at the beginning of the active phase, or 12 h later ($N = 16$). After 16 food restriction cycles, FA was restored to continuous *ad libitum* availability, while the LP cycle continued. Half of the animals in each group was housed with, and the other half without a running wheel. In this protocol, the phases of the conflicting light and FA cues were subsequently in phase, then moving to anti-phase, and back to being in phase, spanning a whole circadian day. Two animals with and three animals without a wheel did not show sufficient circadian activity to be included in the analysis.

Behavioral phasing in the absence of timing cues

Besides the acute influence that FA can have on timing of activity, also the sustained (entrained) effects of FA on timing of activity (both phase and period length) were analyzed in the 16 voles which underwent 8 h of food restriction starting directly after lights off. These voles were again subjected to a $T = 23.5$ h LP cycle, and the same $T = 25$ h food restriction cycle. However, this time, *ad libitum* FA was restored after 7 FA cycles, and at the same time the cycle in LP was discontinued, allowing animals to free-run from the onset of the 8th cycle FA cycle, when the phase difference between food and light availability would have been maximal.

Behavioral analysis

Activity was recorded using Passive Infra-Red detectors and stored in 2-min epochs on a PC. Periodicity of activity during entrained and free-running conditions was assessed using periodogram analysis (Sokolove & Bushell, 1978). For Experiment 1, phases of activity were calculated as a circular Center of Gravity (CG) of activity (Hut et al., 2000; Kenagy, 1980). CGs were calculated according to Mardia (1972) over a 24-h period, starting at each consecutive 2-min epoch in the activity data set

(running CG). An average CG (and linearized SD) was then calculated for each day, with the criterion that a gap of more than 1000 min between two consecutive CGs indicated a transition between two days. As a correction for baseline inter-individual variation within a group, the CG for each individual was corrected for the difference between the individual average CG during first 10 days and the group CG for that period. Data were then expressed as average daily CG and SEM.

For Experiment 2, the contribution of the ultradian components was estimated by smoothing the data over a 3-h window and subtracting the smoothed data from the raw data. The proportions of the behavior belonging to the two components that appear during the desynchronized T-cycles were assessed using a deconvolution approach according to Hiddinga et al. (1997) on the *smoothed* data. In short, the data were folded on a 25-h basis, and the circular average profile was subtracted from the raw data. Periodogram analysis of the residual dataset, containing activity that was not accounted for by the 25-h profile, was used to establish the period of the dominant rhythmic component. The data were then folded on the basis of this second component, and the circular average was subtracted from the raw smoothed data. The residual dataset of this second component was then used to re-assess the period of the first component. This process was iterated three times, at which time the periods of the two rhythmic components were stable. The contribution of the different activity components to the behavior was expressed as the percentage of total variance

All statistical analysis was performed using SAS 9.1, SAS Institute Inc., Cary, NC, USA.

RESULTS

Re-entrainment to a phase advance in light

All voles (N= 42) entrained to the 30-min LP, and periodogram analysis showed entrainment to the light protocol with a mean period of 23.9 ± 0.04 h (AVG $\pm$ SEM). Average phase angles of entrainment (time of lights on - time of corrected CG) were calculated over days 6-10 of the pre-phase advance, and days 6-10 of the post-phase advance period. The phase angles of entrainment are shown in Table 1. Pre-phase shift, phase angles are not different between the treatment groups within the animals housed with or without a running wheel (Proc MIXED, Wheel*Food $P > 0.05$). The phase angles of entrainment are different between animals housed with and without a running wheel before and after the phase-shift (Proc MIXED, Wheel*Light $P < 0.05$), and that difference is more than an hour from the pre-phase shift condition (LSM, $P = 0.05$); post light-shift, the differences are also due to the feeding conditions.

Figure 1 shows the behavioral phases as corrected CG for the different conditions before and after the shift in the light pulse. There are differential responses between feeding and housing conditions to the phase shift in the light (Proc MIXED, Wheel*Food*Light $P < 0.01$). Differences are seen between pre- and post-phase shift in the light, for behavioral phases within a feeding condition, and between feeding conditions during the post-phase shift period. *Ad libitum* fed voles, both those housed with or without a running wheel, show post-phase shift behavioral phases that are intermediate to those of the voles that receive stable or shifting-food restriction (LSM, $P_s < 0.05$ at least). Also similar between voles housed with and without a running wheel, is the enhanced phase advance in behavior when the phase shift in the LP is combined with a similar shift in food restriction (LSM, $P_s < 0.001$), and the absence of a shift in behavioral phase when their food restriction remains at the same time of day (LSM, $P_s > 0.05$).

Voles housed with a running wheel demonstrate a significantly larger behavioral phase advance in response to the phase advance in the light, both in the *ad libitum* and FA availability conditions (LSM, $P_s < 0.05$ at least). In addition to that, in voles that are housed without a running wheel, the behavioral phase is significantly more advanced when the FA advances with the LP (LSM, $P < 0.05$ and $P < 0.0005$ compared to *ad libitum* and stable FA conditions, respectively). In contrast, voles that are housed with a running wheel, show behavioral phases that are significantly less advanced when the FA remains at the same time of day (LSM, $P < 0.05$ and $P < 0.0005$ to *ad libitum* and shift in FA conditions, respectively).

Activity components related to light, food, and running wheel availability

Behavioral phasing in the presence of timing cues

To further investigate the combined effects of running-wheel availability, LP cycles and temporal food restriction, we applied a protocol of desynchronized T-cycles of LP and FA, and applied it to both animals housed with and without a running wheel.

The ultradian component was assessed using a smoothing paradigm, and the percentage of total variance accounted for by this component (see Table 2) was not different between any of the four groups of voles, housed either with or without a running wheel, receiving the first food restriction following the LP or 12 h later (Proc MIXED, $P > 0.05$). Figure 2A shows a representative, double plotted, quantitative actogram of a vole housed with a running wheel receiving the first food restriction directly following the LP. Using a deconvolution method, we assessed the periods of the two major activity components in the circadian range, and calculated the percent of total variance explained by these activity components. The group values for periods and contributions are shown in Table 2, and Figure 2B, 2C, and 2D show the periodograms for the behavioral segments before, during, and after the food restriction cycle for the actogram shown in Figure 2A. The periods of the

two activity components identified were on average 24.14 h (SEM = 0.14 h) for one component, and 25.05 h (SEM = 0.01h) for the other period component. Based on these periods, these components will be referred to below as the shorter- and longer-period components. The periods of these components do not vary within housing condition or within time of first food restriction (Proc MIXED, $P_s > 0.05$ for Wheel and Food). The length of the short-period component is more than 0.5 h longer than that of the LP cycle, while the length of the longer-period activity component is very close to that of the length of the feeding cycle.

The percent of total variance explained by the shorter and longer component (see Figure 2E and 2F) are, in all cases, significantly larger for the longer component (Proc Mixed, Component $P < 0.0001$), and does not vary with housing or feeding condition (Proc Mixed, $P_s > 0.05$ for Wheel and Food). A larger contribution of the longer-period component to the overall activity is also reflected in the higher peak values of the periodogram of that component (Figure 2C).

Behavioral phasing in the absence of timing cues

To assess whether the activity component would continue to be expressed in the absence of the Zeitgebers, we re-ran animals housed with and without a running wheel in the same protocol of the T-cycles of LP and FA, starting the first food restriction immediately after the LP. We stopped both the LP and FA cycles after the 7th FA cycle, so that the last FA and LP timing cues were 180° out of phase. When released in free-running conditions, the period of the voles was 23.73 h (SEM = 0.15), which was not significantly different from the shorter-period component in the preceding experiment (t-test, $P > 0.05$). A number of responses were seen, and Figure 3 shows examples of double-plotted actograms of the four types of responses observed. The actogram in Figure 3A shows a bimodal distribution of activity, and both components have a similar periodicity. The unsmoothed activity profile in Figure 3B again shows a bimodal distribution, but the smoothed data indicate that the longer-period component is phased similar to the feeding conditions and has a larger contribution at first. However, the activity seems to redistribute to a phase more similar to that of

the preceding LP cycle. Actograms in Figures 3C and 3D show only single components in activity. The individual in Figure 3C shows a single activity component with a period and phase similar to that of the preceding FA cycle, while the individual in Figure 3D shows an activity component with a phase similar to that of the preceding LP cycle.

DISCUSSION

Food and running-wheel availability alters the contribution of light to behavioral phasing

We show that timing of food availability can strongly influence activity patterns in the common vole. When adjusting to a phase advance in light entrainment, FA timing that remains at the same time of day results in a strongly attenuated behavioral re-entrainment response. In contrast, when FA is shifted in accordance with the phase advance in light, behavioral re-entrainment is more pronounced.

Running-wheel availability affects re-entrainment to the new LP in two ways. Firstly, the magnitude of the phase advance in behavior is greater in voles housed with a running wheel in both the *ad libitum* and temporally restricted FA conditions. Secondly, post-phase shift the effect of FA, as compared to the *ad libitum* FA conditions, on behavioral phasing is opposite between voles housed with and without a running wheel. While in voles housed without a running wheel the behavioral phase advance is significantly increased by shifting FA together with the LP, in voles housed with a running wheel a significantly decreased behavioral response is seen when FA remains at the same time of day. These effects may be opposite sides of the same coin, and indicate that the impact of light as a timing cue is greater when behavioral organization is more circadian, and that impact of food as a timing cue is more emphasized in ultradian individuals.

This work adds a behavioral equivalent to our previously published work, showing the molecular circadian clock in the liver (which is sensitive to food availability) is inactive in voles with a predominant ultradian behavioral pattern, but is active in voles with circadian organization of behavior (Van der Veen et al., 2006). The result on the relative contribution of the LP and FA in the timing of behavior was most likely also dependent on the protocol. A brief light pulse per cycle may be sufficient for circadian entrainment, but is a weak Zeitgeber compared to a daily 8-h food-

deprivation episode. A single light pulse has been shown to be sufficient to entrain circadian rhythms (DeCoursey, 1972; Pittendrigh & Daan, 1976), in our case the pulse was directed to fall at the end of the inactive phase. It has, however, been shown that multiple light pulses in the dark period can lead to stress in the social vole, *Microtus socialis* (Zubidat et al., 2007). Apart from the single light pulse each cycle, animals were kept in dim red light. Dim light during the dark phase, instead of darkness, has been shown to increase the speed of re-entrainment in hamsters (Evans et al., 2009), and while spectral sensitivity to red light is low, a single red LED in a movement detector has been shown to lengthen the free-running period of mice (Hofstetter et al., 2005).

The arousing effects of cage cleaning may have had an entraining property in our experiment, and may have altered phasing of activity. Phasing of activity in the control group is, however, intermediate to that of either FA groups, indicating that phasing may have been different from non-cage cleaned conditions, but the effects of FA remain. The phasing of the FA cycle may also have had an effect on its relative strength. Kalsbeek et al. (2000) have shown that a 2-h FA during the light phase attenuated re-entrainment of pineal melatonin release to an 8 h phase advance in light in rats. The FA in Kalsbeek's study, however, was given during the light phase and induced activity at a circadian rest phase, while we restricted FA to the dark phase.

How the presence of a running wheel enhances the circadian component in activity is unclear, but is possible it does so without altering the LEO. Running-wheel availability increases the diminished circadian structuring of activity in mice with diminished SCN cellular synchronization without altering transcriptional activity in the SCN (Power et al., 2010). Indeed, in voles, the amplitude of transcriptional activity is decreased under constant dim light, when activity patterns are diminished (Van der Veen et al., 2008). Both seem to indicate that the SCN output is weak in cases where running-wheel availability can alter circadian amplitude. Indeed, we have shown previously that SCN output (vasopressin), but not core output, (measured at the level of VIP) seems

altered in voles with weak rhythms (Jansen et al., 2007) and that the cause of these differences may even lie outside the SCN (Van der Veen et al., 2005).

Light and food can drive separate components of behavior

We observed three components in activity during the combined light and food entrainment paradigm, an ultradian component and two circadian components. Of the circadian components, the component with a period and phasing similar to that of the food cycle was the most dominant. A less dominant component had a period close to 24 h and did not seem to be entrained by the LP. Thus, while the restricted FA seems to act as an entraining signal, light could no longer entrain under these conditions. It has been shown that rats can entrain to multiple Zeitgebers (for reviews see (Mistlberger, 2009; Stephan, 2002)). Similar to our results in the vole, in rats a free-running component in activity, besides a food entrained component, has also been reported in experiments where no light cue was available (Edmunds & Adler, 1977). While the LP was strong enough to entrain activity alone, perhaps when combined with restricted FA the signal is no longer strong enough to entrain. Although the short-period component was not driven by light, its period, initial phase, and co-expression next to the food-related component seem to indicate that it was driven by the LEO.

Light and food entrainable oscillators

The design of these experiments primarily addressed the timing of activity to light and food availability and the role of activity in this process. In the last experiment, we examined whether the activity components could be driven by separate oscillators. When released from their entraining conditions, with food and light in anti-phase, voles showed different behavioral responses. Animals either displayed a bimodal circadian activity distribution, a continuation of the distribution seen

during the entrainment by two timing systems, or only a single component, either originating from the light or from food entrained activity. Some voles in free-running conditions initially showed a dominant, food-related component, and then shifted to a phase similar to the prior shorter activity component.

A food-entrained component in behavior is known to disappear rather quickly in *ad libitum* conditions (Stephan et al., 1979), and Coleman et al. (1982) reported that in rats, several weeks after a FA protocol an activity bout was still discerned at the phase of the former feeding time. When those same rats were given episodes of *ad libitum* food availability this component in behavior disappeared, only to re-appear in a subsequent episodes of food deprivation, although shifting towards and eventually merging with the activity component belonging to the former LP. While rhythms in liver clock genes are normally expressed in mice and rats (Damiola et al., 2000; Stokkan et al., 2001), in voles they are only present under food-restriction cycles or when provided with a running wheel (Van der Veen et al., 2008). The circadian (as opposed to ultradian) feeding pattern induced by the availability of a running wheel and/or restricted food availability likely alters the entrainment of the FEO. The state of this oscillator may well be related to oscillations of the clock in the liver, and also contribute to the effect that food restriction has on phasing of activity in these experiments. In all cases, the SCN is unaffected by restricted FA, unless at the same time this restricts the amount of food consumption (Mendoza et al., 2005). Thus, while temporal food availability is a potent timing cue, it does not normally act at the level of the central pacemaker, but at the level of behavior and peripheral clocks. Although it is uncertain to what degree the food-associated component was driven by an oscillator structure in our experiments, it was able to alter the light-entrained, SCN-driven activity component, both at the levels of phase and period.

In nature, the herbivorous vole is unlikely to be faced with diurnal rhythms in FA, but rather will be exposed to varying predation risks over the day when venturing out to forage (Gerkema, 2002). Both predator activity and the vole response to this predation risk (e.g. social synchronization

of feeding) are dependent on entrained circadian rhythms (Gerkema & Verhulst), and vole ultradian activity patterns show circadian modulation (Gerkema et al., 1993). The common vole is the most widespread Eurasian species of open grasslands (Niethammer & Knapp 1982). In nature the importance of alignment of light-entrained behavior with other external temporal cues will prevail, tuning the ultradian activity patterns to the optimal circadian phase.

Light-entrained activity can be effectively challenged by FA and behavioral modulation. Firstly, we show that running-wheel availability enhances the entraining potential of light and of FA. Secondly, we show that challenging behavior that is driven by a light cycle with a second cycle in FA alters the entrainment to the light cycle. Moreover, our data indicate that the activity component previously entrained to the light, likely is no longer entrained and possibly driven by a free-running light-entrainable clock. In our data, activity has been shown to be a complex of individually driven components, and this activity is, itself, an important modulator of Zeitgeber effectiveness.

Declaration of interest

The authors report no conflicts of interest

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TABLES

Table 1: Phase angles of entrainment pre- and post-phase shift in the light

		Phase angle between light and behavioral phase	
	Food availability	Pre-phase advance Mean $\pm$ SEM	Post-phase advance Mean $\pm$ SEM
No wheel	<i>Ad libitum</i>	-2.40 $\pm$ 0.36 h	-5.33 $\pm$ 0.51 h
	Shifting with light	-2.75 $\pm$ 0.21 h	-7.23 $\pm$ 0.16 h
	Remaining at same time	-2.92 $\pm$ 0.19 h	-2.75 $\pm$ 0.42 h
Wheel	<i>Ad libitum</i>	-1.50 $\pm$ 0.25 h	-1.92 $\pm$ 0.58 h
	Shifting with light	-1.40 $\pm$ 0.17 h	-4.24 $\pm$ 0.42 h
	Remaining at same time	-1.55 $\pm$ 0.22 h	-0.53 $\pm$ 0.45 h

Table 2: Activity components identified under desynchronized T-cycles

	Start of food restriction	Ultradian component % of total variance Mean ± SEM	First circadian component		Second circadian component	
			Period Mean ± SEM	% of circadian variance Mean ± SEM	Period Mean ± SEM	% of circadian variance Mean ± SEM
No wheel	After LP	26.9 ± 4.6 %	25.07 ± 0.02 h	35.0 ± 4.8 %	24.30 ± 0.32 h	12.2 ± 1.8 %
	12 h after LP	21.3 ± 2.6 %	25.01 ± 0.01 h	32.9 ± 9.5 %	24.40 ± 0.44 h	15.3 ± 4.2 %
Wheel	After LP	30.8 ± 3.8 %	25.03 ± 0.02 h	31.2 ± 6.5 %	24.00 ± 0.24 h	15.2 ± 1.2 %
	12 h after LP	30.1 ± 2.6 %	25.06 ± 0.02 h	23.8 ± 4.5 %	23.91 ± 0.20 h	12.5 ± 2.2 %

FIGURE LEGENDS

Figure 1

Relative centers of gravity of activity are shown for the last 5 days before a 4-h phase advance in light (Pre), and days 6-10 after the phase advance in the light (Post). Behavioral phases are shown for animals housed without (right panels), and with (left panels) a running wheel. When an 8-h food-restriction phase advances together with the light, the behavioral phase advance is enhanced in animals housed without a running wheel. The behavioral phase shift to the same phase advance in light is attenuated in voles housed with a running wheel, when food restriction remains at the same time of day. For all three food availability conditions, animals housed with a running wheel show a greater behavioral response to the phase advance in the light than animals housed without a running wheel do. Error bars are SEM, white boxes indicate the 30-min light pulse, grey bars depict the 8-h period when food was absent in the groups that received food restriction. * $P < 0.05$, ‡ $P < 0.005$, # $P < 0.0005$

Figure 2

Double-plotted, quantitative actogram of an individual vole housed with a running wheel during entrainment to a single, 30-min light pulse every 23.5 h (A). Starting on day 11, the vole is subjected to 16 episodes of 8 h food restriction and 17 h food availability ($T = 25$ h); thereafter, food is restored to *ad libitum* availability. Panels B, C, and D show periodograms of behavioral data before, during, and after the food restriction cycles. When both cycles of light and food availability are presented, the total amount of variance explained by the two major circadian activity components is shown for voles housed with (E) and without (F) a running wheel. In panel A, white line indicates the 30-min light pulse, grey bars depict the 8-h period when food was absent.

Figure 3

Example of double-plotted actograms of voles subjected to a single, 30-min light pulse every 23.5 h. Starting on day 12, voles are subjected to 7 episodes of 8 h food restriction and 17 h food availability ($T = 25$ h); thereafter, food is restored to *ad libitum* availability, and the cycle in the light pulses is discontinued. For each of the individuals, the total (left) and above average (right) activity is shown. Actograms are representative for the four different responses observed: A bimodal activity distribution (A), or single activity components (B-D). For the individuals showing a single activity component, phasing may shift (B), or remain at the phase of previous food availability (C) or the light pulse (D) cues. White line indicates the 30-min light pulse, grey bars depict the 8-h period when food was absent.