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Adaptation to short photoperiods augments circadian food anticipatory activity in Siberian hamsters

Sean P. Bradley¹ and Brian J. Prendergast^{1,2}

¹Department of Psychology, University of Chicago, Chicago, IL, 60637 USA

²Committee on Neurobiology, University of Chicago, Chicago, IL, 60637 USA

Abstract

Both the light-dark cycle and the timing of food intake can entrain circadian rhythms. Entrainment to food is mediated by a food entrainable circadian oscillator (FEO) that is formally and mechanistically separable from the hypothalamic light-entrainable oscillator. This experiment examined whether seasonal changes in day length affect the function of the FEO in male Siberian hamsters (*Phodopus sungorus*). Hamsters housed in long (LD; 15 h light/day) or short (SD; 9 h light/day) photoperiods were subjected to a timed-feeding schedule for 10 days, during which food was available only during a 5 h interval of the light phase. Running wheel activity occurring within a 3 h window immediately prior to actual or anticipated food delivery was operationally-defined as food anticipatory activity (FAA). After the timed-feeding interval, hamsters were fed ad libitum, and FAA was assessed 2 and 7 days later via probe trials of total food deprivation. During timed-feeding, all hamsters exhibited increases FAA, but FAA emerged more rapidly in SD; in probe trials, FAA was greater in magnitude and persistence in SD. Gonadectomy in LD did not induce the SD-like FAA phenotype, indicating that withdrawal of gonadal hormones is not sufficient to mediate the effects of photoperiod on FAA. Entrainment of the circadian system to light markedly affects the functional output of the FEO via gonadal hormone-independent mechanisms. Rapid emergence and persistent expression of FAA in SD may reflect a seasonal adaptation that directs behavior toward sources of nutrition with high temporal precision at times of year when food is scarce.

Keywords

energy balance; seasonality; food intake; gonadal hormones; circadian rhythms

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Correspondence to: Sean P. Bradley, Georgia State University, Department of Biology, 50 Decatur St., Natural Science Center, Atlanta, GA 30303, Phone: 404-413-5334, spbradle@gmail.com.

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Introduction

Seasonal changes in physiology and behavior are ubiquitous in nature. Reproduction and immune function, ingestive and social behaviors, and scores of hormones exhibit seasonally-changing phenotypes (reviewed in Prendergast et al., 2009). Day length (photoperiod) figures prominently in the generation and/or entrainment of these seasonal physiological cycles (Goldman, 2001).

In mammals, accurate timing of seasonal phenotypic transitions is dependent on proper entrainment of the circadian system to seasonal changes in day length (Hiebert et al., 2000; Goldman, 2001); the entrained circadian pacemaker in the suprachiasmatic nucleus (SCN), in turn, drives a circadian rhythm in nocturnal pineal melatonin secretion (Borjigin et al., 2012). Thus, seasonal timing is dependent on feed-forward information from the circadian system. Seasonal physiological adaptations may also exert feedback effects on the circadian system. Following adaptation to short, winter-like day lengths (SDs), male Syrian hamsters exhibit larger light-induced phase shifts of the circadian clock as compared to hamsters housed under long day (LD) photoperiods (Pittendrigh and Daan, 1976; Pittendrigh et al., 1984). The photic thresholds for circadian phase-resetting are approximately 40 times greater in LD relative to SD hamsters (Glickman et al., 2012), suggesting that day length alters the sensitivity of the SCN to photic input, and consistent with a model in which the amplitude of the underlying circadian pacemaker is greater in LD relative to SD (Evans et al., 2004; Glickman et al., 2012).

In addition to light, non-photoc time cues (zeitgebers; e.g., temperature, social interactions, food availability) can also influence the circadian system (Mrosovsky & Salmon, 1987; Mrosovsky, 1996). However, the extent to which photoperiod alters circadian responses to non-photoc zeitgebers has received limited study. In castrated male Syrian hamsters, the magnitude of circadian phase shifts induced by exposure to a novel running wheel were comparable under LD and SD photoperiods. Among the diverse non-photoc zeitgebers, however, food is among the most potent (Mistlberger, 2011). The timing of food availability can set the phase of circadian activity and body temperature rhythms, even in the presence of a conflicting light-dark cycle (Coleman et al., 1982). Feeding manipulations that limit food access to a single, brief (3–5 h) timed daily meal yield robust food anticipatory activity (FAA), which stably precedes the timing of the meal (Mistlberger, 2011). Following the discontinuation of timed feeding, FAA persists for several cycles under conditions of total food deprivation, consistent with the conjecture that a self-sustained, but rapidly-dampening, food entrainable oscillator (FEO), or network of oscillators, including the SCN (Mistlberger, 2011), participates in the generation of circadian FAA. Whether, in common with other non-photoc zeitgebers, entrainment to food is likewise unaffected by seasonal changes in day length remains largely unexamined. Food entrainment persists following SCN ablation (Stephan et al., 1979) indicating that photoperiod information is not required for entrainment to food; however, in addition to driving the timing of feeding behavior, the SCN exerts temporal gating of FAA and affects FAA amplitude (Acosta-Galvin et al., 2011), leaving open the possibility that photoperiod-driven changes in the amplitude of the circadian oscillation in the SCN may influence the expression of FAA.

Seasonal plasticity in the expression of FAA may afford hamsters additional mechanisms for maintaining energy balance. Enhancements in the expression of FAA in winter, for example, might direct temporally-precise behavior toward sources of nutrition at times of year when food is scarce. Thus, the present study tested the hypothesis that seasonal changes in photoperiod alter the ability of timed feeding to induce FAA. Experiments examined the rate of induction of FAA, FAA amplitude, and the persistence of FAA following the cessation of timed feeding schedules in adult male Siberian hamsters. FAA has not been directly examined in this species previously; however, Siberian hamsters are a canonical model for examining photoperiodism, and may prove useful in examinations of photoperiodic regulation of FAA. Hamsters exhibit robust photoperiodic changes in circadian entrainment (Prendergast & Pyter, 2009), food intake (Bartness, 1996), and, unlike Syrian hamsters, readily ingest food during the light phase (Paul et al., 2004). Because gonadal hormones affect food intake in this species (Bartness, 1996), a follow-up study was also performed to test the hypothesis that photoperiodic changes in FAA are mediated by the withdrawal of gonadal hormones that hamsters exhibit following phenotypic adaptation to short photoperiods.

Materials and Methods

Animals and housing

Siberian hamsters (*Phodopus sungorus*) in all studies were derived from a breeding colony maintained at the University of Chicago on a long day (LD) photoperiod (15 h light, 9 h dark; lights off at 18:00 CST). Hamsters were housed in polypropylene cages (28x17x12 cm) containing wood shaving bedding (Sani-Chips, Harlan) along with cotton nesting material. Food (Teklad 8604, Harlan) and filtered tap water were provided ad libitum except during intervals of timed feeding, described below. In addition, during intervals of timed feeding, a nutritive supplement (Nutri-cal; Vetoquinol) was provided alongside the food. Ambient temperature was maintained at 20±2°C with relative humidity 50±5%. All treatments conformed to the USDA Guidelines for the Care and Use of Laboratory Animals and received prior approval by the local Animal Care and Use Committee.

Experiment 1: Effects of photoperiod on entrainment to food

At the start of Experiment 1, adult (90–120 day old) male Siberian hamsters (n=72) from the 15L breeding colony were housed 1/cage and either transferred to a short day photoperiod (SD; 9 h light, 15 h dark; lights off at 18:00 h CST; n=36) or remained in their natal LD (n=36). After 12 weeks of exposure to photoperiods (week 12), reproductive condition in all hamsters was assessed under light isoflurane anesthesia, via a measure of estimated testis volume (ETV; testis length * testis width²), which correlates positively ($R^2 > 0.9$) with testis weight (Gorman & Zucker, 1995). A minority of Siberian hamsters fails to undergo gonadal regression in SD ('nonresponders'; Prendergast et al., 2001); thus only SD hamsters with ETV < 300 on week 12 (n=27) were included in subsequent analyses. ETVs on week 12 were significantly greater in LD relative to SD hamsters (mean ±SEM; LD: 433±12; SD: 96±11; $p < 0.001$, data not illustrated).

Timed feeding procedure

On week 12, hamsters were transferred to larger polypropylene cages (26x48x21 cm) equipped with stainless steel running wheels (11.5 cm diameter) and allowed 7 days to acclimate. Running wheels were always present in the cage. The timing of events during timed feeding and control treatments are illustrated in Fig. 1.

Following running wheel acclimation for 7 days, experimental hamsters were trained on a timed feeding (TF) paradigm (LD: n= 17; SD: n=17); control hamsters remained on ad libitum (AL) access to food (LD: n= 18; SD: n=17; Fig. 1). During TF training, food was only available during a 5 h interval which began 6 h prior to lights off. TF training was maintained for 10 days, after which hamsters were subjected to 2 days of ad libitum feeding, followed by a 2 days of total food deprivation (TFD) to assess FAA (FAA early probe trial). Following the early probe trial, hamsters were returned to ad libitum food access for 7 days (AL interval), after which another 2 day interval of TFD occurred to assess FAA retention (FAA late probe trial).

Home cage locomotor activity collection

Running wheels were fitted with 2 small magnets that recorded half-wheel revolutions via a magnetic reed switch. Switch closures were collected by a hardware interface (Colburn Instruments, Allentown, PA) linked to an automated circadian data collection software system (Clocklab; Actimetrics Inc., Evanston, IL). Wheel activity was sampled continuously and acquired in 6 min bins.

Quantification of food anticipatory activity

By convention (Pitts et al., 2003), activity occurring during the 3 h immediately prior to the actual (during TF training treatments) or expected (during ad lib and TFD [early and late] treatments) delivery of food was coded as FAA. To correct for individual differences in wheel running levels, FAA was expressed as a proportion of total daily activity for each hamster (%FAA), as the quotient of the total number of wheel revolutions during the 3 h FAA interval divided by the total number of wheel revolutions during the preceding 24 h (Pitts et al., 2003). %FAA was calculated separately for each day of the experiment. For statistical comparisons, during the early and late TFD intervals, FAA data were collapsed across each 2 day testing window to generate a single FAA value; likewise, data were collapsed across the entire 7 day AL interval.

The timing of the first bout of daytime FAA was also determined for each experimental day. Specifically, each 6 minute activity bin was coded as above or below the 20th percentile of locomotor activity for that day and the resulting data were convolved with a 3 h function representing constant activity (in Clocklab). The onset of FAA was defined as the time of the first local maximum value falling above the average value of the convolution (Prendergast and Pyter, 2009).

Experiment 2: Effect of gonadal hormones on entrainment to food

Siberian hamsters exhibit gonadal regression and marked decreases in gonadal hormone secretion following adaptation to SD (Lerchl et al., 1993); therefore, a second experiment

investigated the effects of withdrawal of gonadal hormones on FAA. Adult male hamsters ($n=36$) from the LD breeding colony were individually housed in small (28x17x12 cm) polypropylene cages as described in Experiment 1, with ad libitum access to food and water. Hamsters were bilaterally gonadectomized under isoflurane anesthesia (GnX; $n=18$) or received a sham-gonadectomy surgery (Sham; $n=18$), according to procedures described elsewhere (Prendergast et al., 2008). Briefly, testicular blood vessels were cauterized and the testes removed through a midline incision; the interior incisions were closed with vinyl sutures, and the skin was repaired with stainless steel surgical staples. Sham operations repeated all procedures without cauterizing or externalizing the testes. Hamsters received 0.1 mg/kg buprenorphine injections every 12 h for 48 h after surgery to alleviate postsurgical discomfort.

Six weeks after GnX and Sham procedures, hamsters were transferred to larger running wheel cages for 7 days of acclimation, after which they received TF training, ad lib feeding, and TFD probe trials, identical to those described in Experiment 1.

Statistical analyses

The effects of feeding condition, gonadectomy, and photoperiod condition on the expression of FAA were analyzed using repeated-measures ANOVA for longitudinal assessments and factorial ANOVAs for aggregate measures. Pairwise comparisons were conducted using Fisher's PLSD after a significant omnibus ANOVA result. For all analyses, observed differences were considered significant if $p \leq 0.05$. All statistics were performed using Statview 5.0 (SAS Institute).

Results

Experiment 1. Effects of photoperiod on acquisition and retention of FAA

Representative examples of locomotor activity during a course of TF training are depicted in Figure 2. During the 10 d TF training interval, FAA was evident in both LD and SD hamsters ($F_{10, 940} = 20.4$, $p < 0.001$; Fig. 3A), but photoperiod interacted with time to affect the pattern of FAA emergence ($F_{10, 940} = 7.2$, $p < 0.001$; Fig. 3A). In SD hamsters, FAA was evident after 1 d of TF training ($p < 0.01$), whereas in LD hamsters significant FAA emerged only after the third day of TF training ($p < 0.05$). In addition, the absolute amount of FAA observed in SD hamsters was significantly greater than that of LD hamsters from days 1 through 10 of the TF training period ($p < 0.01$, all comparisons; Fig. 3A). Because baseline activity was greater in SD relative to LD hamsters under AL conditions ($p < 0.05$, all comparisons), we performed an additional analysis that corrected for spontaneous activity levels: mean % FAA exhibited on each day by control hamsters was subtracted from % FAA on the same day exhibited by hamsters undergoing feeding manipulations. This yielded a measure of 'baseline-subtracted FAA' (Fig. 3B). Although no main effect of photoperiod was evident on baseline-subtracted FAA ($p > 0.05$), photoperiod interacted with time to affect the pattern of change in this measure ($F_{1, 10} = 4.89$, $p < 0.001$).

Beyond the emergence of FAA, the circadian distribution of wheel running activity also changed following the onset of TF treatments. The proportion of total daily activity

occurring during the dark phase decreased precipitously over the course of TF treatments ($F_{9,288}=7.24$, $p < 0.0001$; Fig. 4A), and after 10 days of TF treatment, approximately 40% of total activity was occurring outside of the dark phase. Photoperiod did not affect the pattern of change in nocturnal activity ($F_{9,288}=0.59$, $p > 0.80$), however, consistent with the earlier emergence of FAA in SD, significant decrements in nocturnal activity were first evident in SD on day 3 of TF treatment but did not appear until day 5 in LD hamsters.

Photoperiod influenced the time of FAA onset ($F_{5,100} = 13.1$, $p < 0.01$; Fig. 4B). During the final 2 days of the TF training interval (days 9 and 10), FAA began at a comparable time of day in both LD and SD hamsters ($p > 0.05$, both comparisons). However, during the subsequent 2 days of ad libitum feeding, and on the first day of the TFD early probe trial, the onset of FAA in SD hamsters was significantly advanced in phase relative to that of LD hamsters ($p > 0.05$, all comparisons; Fig. 4B).

Analysis of data collapsed across the 10 day TF training period revealed that both photoperiod ($F_{1,47} = 26.25$, $p < 0.001$) and feeding manipulations ($F_{1,47} = 56.22$, $p < 0.001$) affected FAA (Fig. 5A). Overall, SD hamsters exhibited substantially greater levels of FAA relative to LD hamsters ($p < 0.01$).

In the TFD early probe trial, both LD and SD hamsters exhibited significant FAA (Fig. 5A). Photoperiod ($F_{1,47} = 30.56$, $p < 0.001$) and feeding manipulations ($F_{1,47} = 28.2$, $p < 0.001$) each affected FAA. Levels of FAA were >50% greater in SD relative to LD hamsters ($p < 0.001$) during this interval.

During the subsequent 7 day ad lib interval, an interaction between photoperiod and feeding manipulations affected FAA ($F_{1,47} = 10.51$, $p < 0.01$; Fig. 5A). FAA persisted during this interval in SD hamsters ($p < 0.001$ vs. ad lib fed groups; $p < 0.001$ vs. LD-TF group), but FAA was absent LD hamsters ($p > 0.05$ vs. LD-ad lib-fed group).

Lastly, during the TFD late probe trial, a significant interaction between photoperiod and timed feeding was evident ($F_{1,47} = 12.409$, $p < 0.001$; Fig. 5A). FAA magnitude was over 3-fold greater in SD relative to LD ($p < 0.001$).

Locomotor activity levels of AL-fed hamsters was greater in SD than LD during each post-TF experimental epoch ($p < 0.05$ all comparisons; Fig. 5A). Thus, we also calculated 'baseline-subtracted FAA' (see above, Fig. 3) for the TFD early-probe, *ad lib*, and TFD late-probe assessment intervals (Fig. 5B). Baseline-subtracted FAA did not differ between LD and SD hamsters during the 10 days of TF training or during the 2 day TFD early probe trial ($p > 0.05$, both comparisons; Fig. 5B; see also Fig. 3B). However, baseline-subtracted FAA was significantly greater in SD relative to LD hamsters during the 7 day ad lib interval ($p < 0.01$) and during the TFD late probe trial ($p < 0.05$; Fig. 5B).

Experiment 2. Effects of gonadal hormones on acquisition and retention of FAA

During the TF training period, FAA emerged in both GnX and Sham hamsters ($F_{10,420} = 4.3$, $p < 0.001$; Fig. 6). However, GnX did not significantly affect the pattern of FAA emergence ($F_{10,420} = 1.31$, $p > 0.05$). Significant FAA was first evident by the fourth day of

TF training in GnX hamsters and by the sixth day in Sham hamsters ($p < 0.05$ vs. AL, both comparisons). FAA did not differ between GnX and Sham hamsters on any day of TF training ($p > 0.05$, all comparisons). Among ad lib-fed hamsters, activity during the 3 h FAA window was comparable between GnX and sham-operated hamsters ($p > 0.05$, all comparisons; Fig. 6).

Castration did not affect FAA during any subsequent stage of the experiment (TFD early probe trial: $F_{1,68} = 0.294$, $p > 0.05$; ad lib interval: $F_{1,68} = 1.47$, $p > 0.05$; TFD late probe trial: $F_{1,68} = 0.01$, $p > 0.05$; Fig. 7).

Discussion

Male Siberian hamsters exhibited robust entrainment of locomotor activity to timed feeding during the light phase. In both LD and SD photoperiods, FAA appeared within 10 days of the initiation of TF and re-emerged under food deprivation 2 days after the discontinuation of TF (early probe). Persistent FAA under food deprivation is considered evidence for the output of a self-sustained, food-entrainable oscillator (FEO; Mistlberger, 1994). FAA was also evident during a later (>1 week post-TF) food deprivation probe trial, but only in SD hamsters. During TF training and during both probe trials, the absolute magnitude of FAA was significantly greater in SD hamsters, but spontaneous light-phase activity levels among control-fed hamsters were also greater in SD. However, even when this elevated non-specific daytime activity was taken into account, greater and more persistent FAA was evident in SD. Together, the data indicate that short days augment the expression of FAA, which suggests that changes in photoperiod alter the substrates that constitute the FEO. In common with the effects of light on the light-entrainable oscillator, the effects of timed feeding on the central and/or peripheral substrates that comprise the FEO are enhanced under short photoperiods; this stands in contrast to the relative insensitivity of the circadian system of Syrian hamsters to photoperiodic modulation of responses to other non-photoc zeitgebers (e.g., novel wheel access; Evans et al., 2004).

A principal outcome of this study was the observation that FAA was enhanced in SD during the two probe trials. However, SD hamsters also exhibited robust FAA during the week of *ad libitum* feeding that followed initial TF training (Fig. 5B). In many studies of food entrainment, FAA rapidly disappears during periods of ad libitum feeding and only emerges during periods of food deprivation (Mistlberger, 1993, 1994; Stephan, 1997). Persistent FAA under *ad libitum* feeding provides further evidence consistent with enhanced entrainment to food following adaptation to SD.

During TF, all hamsters received food for 5 h, terminating 1 h prior to the onset of darkness. However, the use of different photoperiods in the present experiment necessitated delivering food at different ZTs in LD and SD hamsters. Specifically, food appeared 9 h after lights on in LD hamsters (ZT 9 - ZT 14; late subjective day), whereas it appeared earlier in subjective day (3 h after lights on; ZT 3 - ZT 8) in SD hamsters. In rats, phase relations between food appearance and light onset alter the expression of FAA: timed feeding later in subjective day leads to more robust FAA, as compared to early subjective day (Stephan, 1986). It seems unlikely that such a mechanism contributes substantially to the outcome described here.

Because the duration of the light phase in SD was only 9 h, SD hamsters had access to food during a broad interval of time, ranging from mid-to-late subjective day, whereas in LD hamsters, food access occurred relatively later in subjective day. If TF occurring later in subjective day augmented FAA, then this should have resulted in greater FAA in LD hamsters. The present data suggest that subjective-day-dependent effects of TF as elaborated in rats (Stephan, 1986) are unlikely to be operant in Siberian hamsters.

Three non-exclusive mechanisms may account for the enhanced expression of FAA under SD: (1) increases in the strength of output signals that drive FAA, (2) decreases in the activity of mechanisms that inhibit the expression of FAA, and (3) increased stimulus strength (i.e., the salience of food as a zeitgeber). FAA has a complex genesis, and the FEO is likely a distributed neural system. Recent work suggests that the expression of FAA reflects the net interaction between neural and peripheral substrates that entrain to the timing of food, and the SCN, which inhibits daytime activity (Acosta-Galvan et al., 2011). The substrates that drive FAA output have proven challenging to localize neuroanatomically (Acosta-Galvan et al., 2011), but cannot be excluded as a potential target of SD effects in the present study. Neural pathways that inhibit daytime locomotor activity-- and thus oppose the expression of FAA-- include the SCN. Removal of SCN-mediated inhibition on daytime activity via lesions augments the expression of FAA in rats (Acosta-Galvan et al., 2011). Similar augmentation via disinhibition may be occurring under SD photoperiods. Enhanced phase resetting responses to light in SD relative to LD (Pittendrigh et al., 1984; Evans et al., 2004), and a broader range of circadian time over which light is capable of inducing phase shifts in SD (Pittendrigh et al., 1984), collectively suggest that the amplitude of the circadian pacemaker is dampened following entrainment to SD. In Siberian hamsters, entrainment to SD markedly reduces the amplitude of the circadian locomotor activity rhythm, and disinhibits light-phase locomotor activity under *ad lib* feeding conditions (Prendergast & Zucker, 2012; Prendergast et al., 2013; present study). In addition, the amplitude of the circadian rhythm in the expression of mRNA of some clock genes (e.g., *per2*) is significant lower in SD (Johnston et al., 2005). A reduction in the inhibition of light-phase activity could account for the enhanced FAA in SD, independent of any photoperiodic increases in FAA output. Entrainment of the circadian system to SD, and the resultant decrease in pacemaker amplitude, may diminish SCN inhibition of light-phase activity, and thereby disinhibit the expression of FAA in the presence of appropriate feeding cues.

Overt aspects of appetitive and food-driven behavior in Siberian hamsters seem unlikely to explain the present data. Micronutrient selection changes in response to photoperiod in this species: SD hamsters prefer a diet higher in protein and carbohydrates, and lower in fat, relative to LD hamsters (Fine & Bartness, 1996). Siberian hamsters are avid food hoarders, but do not markedly alter the size of their food hoards in SD relative to LD. (Wood & Bartness, 1996): baseline food hoarding was comparable in LD- and SD- housed male Siberian hamsters (Wood & Bartness, 1996). Furthermore, a brief interval of food deprivation is a potent trigger for increasing food hoarding, but during both the early (5–6 weeks; Teubner & Bartness, 2009) and the later (11 weeks; Wood & Bartness, 1996) intervals of exposure to SD, deprivation-induced food hoarding responses were comparable

in SD and LD hamsters. Enhanced FAA in SD may reflect a novel aspect of photoperiod-dependent changes in food-motivated behavior in this species (cf. Bartness et al., 2011).

Whether changes in stimulus strength (i.e., the salience of food as a zeitgeber) also contribute to enhanced FAA in SD is not clear. A mechanistic examination of photoperiodic differences in the role of orexigenic peptides in the induction of FAA was beyond the scope of the present study, but differences in the neuroendocrine sequelae of acute food deprivation also appear unlikely to explain the observed effect of photoperiod on FAA. For example, episodic ghrelin signaling is sufficient to drive FAA in rats (Merkestein et al., 2012), but photoperiod does not affect ghrelin responses to food deprivation in Siberian hamsters (Tups et al., 2004b). Changes in sensitivity to ghrelin also seem unlikely to explain different FAA in SD compared to LD: consummatory responses to ghrelin are markedly greater in LD relative to SD (Bradley et al., 2010). If consummatory responses to orexigenic peptides are predictive of the salience of these peptides in inducing FAA, then it appears unlikely that photoperiodic changes in the sensitivity of the FEO to ghrelin input mediates enhanced FAA responses to timed feeding in SD. Photoperiodic changes in leptin signaling also seem unlikely to play a role in seasonal changes in the expression of FAA. LD hamsters are resistant to the catabolic effects of leptin (Atcha et al., 2000; Klingenspor et al., 2000; Rousseau et al., 2002; Tups et al., 2004a), and FAA is markedly enhanced in *ob/ob* mice and eliminated by chronic treatment with leptin (Ribeiro et al., 2011). If, in common with mice, hypothalamic leptin signaling were antagonizing FAA in Siberian hamsters, then one would predict augmented FAA in LD.

Adaptation to SD triggers gonadal regression in Siberian hamsters, and accompanying this regression is a marked decline in gonadal hormone secretion (Lerchl et al., 1993; Prendergast et al., 2006). Gonadal hormones alter food intake in a number of species, including *Phodopus* (Bartness, 1996); thus, in experiment 2, we examined whether the withdrawal of gonadal hormones alone was sufficient to mimic effects of SD on FAA. Castration did not enhance FAA in LD hamsters, indicating that withdrawal of gonadal hormone secretion in SD is not sufficient to impart the SD FAA phenotype. Although the artificial removal of gonadal hormone secretion was not sufficient to mimic the strong FAA observed in SD hamsters, gonadal hormones may still affect the acquisition of FAA under physiological conditions, perhaps by regulating food intake (Wade, 1972) or by influencing responsiveness to changes in available nutrition (Hoyenga and Hoyenga, 1982; Wade and Gray, 1979).

Previous reports have described food availability as a particularly potent non-photic zeitgeber; in contrast with other non-photic cues, timed feeding determines the phase of clock gene rhythmicity in peripheral tissues such as the kidney, lung, and liver, as well as select hypothalamic nuclei (Gooley et al., 2006; Pezuk et al., 2010). Whether the absence of photoperiodic modulation of responsiveness to non-photic zeitgebers extends to food zeitgebers was the primary focus of this study. The present data describe robust effects of photoperiod on the expression of FAA, and thus underscore the uniqueness of food as a non-photic zeitgeber. Increased sensitivity of the circadian system to food-related cues, but not other non-photic cues, in SD may reflect a seasonally-changing strategy for maintaining energy balance.

In summary, the present report documents enhanced FAA under SD photoperiods, and excludes the hypothesis that decreases in gonadal hormone secretion are sufficient to mediate this seasonal change in a learned behavior. Food availability may represent a uniquely salient stimulus and may engage mechanisms of entrainment different from other non-photic zeitgebers (Mistlberger, 2011). Adaptation to SD incurs a constellation of changes in behavior and physiology that collectively may permit hamsters to survive seasonal intervals of low ambient temperatures and food scarcity.

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Highlights

- Hamsters in long (LD) and short day lengths (SD) received daily timed meals
- Food anticipatory activity was greater and persisted longer in SD
- Castration in LD did not augment FAA, suggesting gonadal hormone-independence
- Seasonal plasticity in the circadian system facilitates FAA in winter

Figure 1.

Schematic representation of behavioral interventions to induce FAA. Timed feeding (TF) training was imposed for 10 days, followed by 2 days of *ad libitum* (AL) feeding, 2 days of total food deprivation (TFD; early probe). Food was restored ad libitum for 1 week after the early probe, and then a second interval of TFD for 2 days (later probe) was performed. Control hamsters had AL access to food for the entire experiment except during the 2 day TFD intervals. Food anticipatory activity (FAA) was quantified during all intervals as wheel running activity occurring during the 3 h prior to the onset of actual and/or anticipated food delivery.

Figure 2. Representative double-plotted actograms of male Siberian hamsters in Experiment 1. Siberian hamsters were housed in LD (A-D) or SD (E-H) for 12 weeks, after which a timed feeding regimen was implemented. Shaded regions indicate intervals when food was available. Rectangular bins indicate the 3 h interval of activity coded as food anticipatory activity (FAA). So as not to obscure the data, annotations appear only on one side of the double-plotted actogram.

Figure 3.

Mean $\pm$ SEM (A) food anticipatory activity (FAA) expressed as a percentage of total daily activity and (B) baseline-subtracted FAA of Siberian hamsters in long (LD; $n = 36$) or short (SD; $n = 27$) day lengths subjected to a timed-feeding regimen. Data in panel A depict FAA during the initial 10 days of TF training ('training'), the 2 days of recovery on *ad libitum* feeding ('ad lib'), and the 2 day total food deprivation (early) probe trial ('TFD'). * $P < 0.05$ SD-timed fed vs. LD timed fed. Panel B depicts only the TF training interval. Black bars

along abscissa designate experimental days on which TF value differed significantly ($P < 0.05$) from *ad libitum* value, within photoperiod.

Figure 4.

Mean $\pm$ SEM (A) nocturnal activity expressed as a percentage of total daily activity during the 10 days of timed feeding and (B) onset of daytime locomotor activity of Siberian hamsters in LD and SD subjected to a timed-feeding regimen. Data in panel B depict the final 2 days of TF training ("TF training"), the 2 days of recovery on *ad libitum* feeding ("ad lib"), and the 2 day total food deprivation (early) probe trial ("TFD"). Dashed vertical lines indicate FAA window, shaded region indicates time of food availability, and black background indicates the scotophase. * $P < 0.05$ vs. LD value.

Figure 5.

(A) Mean + SEM food anticipatory activity (FAA) expressed as a percentage of total daily activity of Siberian hamsters in LD and SD subjected to a timed-feeding regimen. Data depict mean FAA collapsed across the entire 10 days of TF training ('Timed Feeding Training'), the 2 day total food deprivation (early) probe trial ('TFD Early Probe'), the 7 days of *ad libitum* feeding that followed the early probe trial ('ad libitum'), and the final 2 day total food deprivation (late) probe trial ('TFD Late Probe'). * $P < 0.05$ vs. ad lib value, within photoperiod. † $P < 0.05$, SD-timed fed vs. LD-timed fed value. (B) Mean + SEM FAA values following correction for group differences in spontaneous locomotor activity (baseline subtraction; see *Results*) of Siberian hamsters in LD and SD subjected to a timed-feeding regimen. Abbreviations and conventions as in Panel A. * $P < 0.05$ vs. LD value.

Figure 6.

(A) Mean $\pm$ SEM food anticipatory activity (FAA) expressed as a percentage of total daily activity of gonadectomized GnX; n=18) or sham-operated (Sham; n=18) Siberian subjected to a timed-feeding regimen. Data depict FAA during the initial 10 days of TF training ('training'), the 2 days of recovery on *ad libitum* feeding ('ad lib'), and the 2 day total food deprivation (early) probe trial ('TFD'). (B,C) Representative double-plotted actograms of sham-operated (B) and gonadectomized (C) male Siberian hamsters in Experiment 2. Hamsters were subjected to timed feeding in LD (15h light/day). Shaded regions indicate

intervals of food availability. Rectangular bins indicate the 3 h interval of activity coded as food anticipatory activity (FAA). So as not to obscure the data, annotations appear only on one side of the double-plotted actogram. * $P < 0.05$, TF vs. control, GnX; † $P < 0.05$, TF vs. control, Sham.

Figure 7.

Mean +SEM food anticipatory activity (FAA) expressed as a percentage of total daily activity of sham-operated (Sham) and gonadectomized (GnX) Siberian hamsters subjected to a timed-feeding regimen in LD. Data depict mean FAA collapsed across the entire 10 days of TF training ('Timed feeding Training'), the 2 day total food deprivation (early) probe trial ('TFD Early Probe'), the 7 days of *ad libitum* feeding that followed the early probe trial ('*ad libitum*'), and the final 2 day total food deprivation (late) probe trial ('TFD Late Probe'). *P<0.05 vs. ad lib value, within surgical condition.