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Oscillators entrained by food and the emergence of anticipatory timing behaviors

Rae SILVER^{1,2} and Peter BALSAM¹

¹ Department of Psychology, Barnard College, New York City, New York, USA

² Department of Psychology, Columbia University, New York City, New York, USA

Abstract

Circadian rhythms are adjusted to the external environment by the light–dark cycle via the suprachiasmatic nucleus, and to the internal environment of the body by multiple cues that derive from feeding/fasting. These cues determine the timing of sleep/wake cycles and all the activities associated with these states. We suggest that numerous sources of temporal information, including hormonal cues such as corticoids, insulin, and ghrelin, as well as conditioned learned responses determined by the temporal relationships between photic and feeding/fasting signals, can determine the timing of regularly recurring circadian responses. We further propose that these temporal signals can act additively to modulate the pattern of daily activity. Based on such reasoning, we describe the rationale and methodology for separating the influences of these diverse sources of temporal information. The evidence indicates that there are individual differences in sensitivity to internal and external signals that vary over circadian time, time since the previous meal, time until the next meal, or with duration of food deprivation. All of these cues are integrated in sites and circuits modulating physiology and behavior. Individuals detect changes in internal and external signals, interpret those changes as “hunger,” and adjust their physiological responses and activity levels accordingly.

Keywords

circadian timing system; food-entrainable oscillators; ghrelin; insulin; interval timing; stomach

HISTORICAL CONTEXT: CIRCADIAN OSCILLATORS ENTRAINED BY FOOD

For survival, it is important for organisms to maintain internal equilibrium. The competing demands of life, including eating, sleeping, avoiding predators, and finding mates require that physiological processes are temporally adjusted to occur at optimal times. This need also occurs at the level of individual cells of the body as they too must partition the timing of anabolic and catabolic functions. Understanding of the temporal tuning of this system leapt forward with studies of the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN, viewed as the master circadian clock in the brain, was first discovered in 1972. At that time, the importance of photic input in setting the phase of the brain clock, and the role of the SCN in regulating circadian rhythmicity in both locomotor activity and adrenal corticosterone, were described.^{1–3}

However, even before the identification of the SCN, the notion that food-related signals are important in the mammalian circadian timing system was suggested in Richter’s observation

that rats fed one meal a day increased their daily locomotor activity prior to mealtime.⁴ Very soon after the role of the SCN in circadian control was discovered, it was demonstrated that daytime restriction of food and water phase-shifted the circadian rhythm of plasma corticoids and body temperature and that these rhythms persisted in SCN-lesioned animals subject to restricted food access.^{5,6} Phase-setting responses to restricted daily feeding were placed in two categories: driven or anticipated (see table 1 in reference⁷). In some cases, exemplified by drinking behavior, synchronization was a product of direct, exogenous influences of food intake. In other cases, exemplified by locomotor activity and lever pressing, synchronization was attributable to a food-entrained circadian mechanism. Importantly, most anticipatory circadian rhythms persist in SCN-lesioned animals under conditions of temporally restricted food access (reviewed in references^{8–10}). In summary, it is clear that the control of behavioral and physiological responses that constitute food-anticipatory activities (FAAs) are not dependent on the SCN, and their regulation is an intense focus of current research.¹¹

The discovery of circadian clock genes^{12,13} and their cycling in the SCN permitted the examination of the cellular basis of these numerous circadian rhythms. Initially it was thought that cellular clocks were restricted to SCN neurons, but the finding of circadian oscillation in cultured fibroblasts heralded a tremendous shift in the conceptualization of the circadian timing system.¹⁴ Specifically, the broader significance of circadian oscillations in fibroblasts quickly came into focus with the discovery of cell-based circadian clocks and clock genes in numerous peripheral tissues including the lung, liver, heart, etc.^{10,15–18} In this context the role of feeding/fasting-related temporal signals was re-examined with the discovery that in all peripheral tissues studied, circadian rhythms are entrained by food-derived cues inherent in schedules of feeding–fasting. Such findings raised salient questions about the nature of phase-setting signals.

There is widespread agreement that individual cells in numerous tissues contain circadian clocks. The SCN itself is comprised of a network of cellular oscillators that together function as a “master clock.”^{19,20} Cellular oscillators occur in numerous extra-SCN brain sites and in peripheral glands and organs throughout the body (reviewed in references^{21,22}). A key question is how interactions among these SCN and extra-SCN light- and food-entrained oscillators (FEOs) give rise to anticipatory responses at the level of individual glands and tissues, and at the level of the behavior.

FROM CELLS TO GLANDS AS READOUTS OF ANTICIPATORY RESPONSES

A useful heuristic in contemplating the circadian timing system has been to view it as comprised of three components: input-clock-output. The distinct components of inputs–clocks–outputs of these multiple peripheral oscillators are not known precisely. In mammals most central and all peripheral oscillators lack direct photic inputs, and their phases are generally determined, directly or indirectly, by the SCN. However, when cues derived from photic- and food-related signals are in conflict, such as occurs when food access is temporally restricted to the organism’s inactive phase, peripheral oscillators are synchronized by food-derived signals, rather than by SCN-derived signals (reviewed in references^{10,23}). The nature of the phase-setting inputs, clock genes, output signals, and mechanisms of integration of the numerous outputs of central and peripheral cell-based oscillators are accessible to experimental analysis.

Some aspects of the response of cellular clocks are unique to specific tissues. In a study of FAA in which *Per1* rhythms were compared in various organs of the *Per1* luciferase transgenic rat, there were differences in the time course of phase-shifting responses.¹⁶

Animals were entrained to daytime restricted feeding, then fed *ad libitum* and subsequently deprived. Tissues from various organs were removed and bioluminescence was imaged over days. The results revealed diversity in the responses among peripheral oscillators. The stomach was less stably entrained than other tissues such as liver. The transients following termination of restricted feeding moved in the advance direction in liver, but in the delay direction in gastrointestinal tissues. Several other parameters also differed among tissues, including damping rate, phase position, and rhythm amplitude.

Another indication of differences to input signals among peripheral tissues is seen in a lovely set of parabiosis experiments in which mice bearing SCN lesions were yoked to intact mice. Rhythmic clock gene expression is detected in the lesioned animal in the liver and kidney, but not in the heart, spleen, or skeletal muscle.²⁴ The results provide definitive evidence of differences among tissues in effectiveness of phase-setting signals, and indicate that non-neural signals, possibly blood-borne, are sufficient to maintain circadian rhythms of clock gene expression in some tissues.

Of specific interest here is the subset of oscillators that produce systemically circulating signals that are phase-shifted by food and are secreted *prior to* regularly timed meals. These FEOs produce hormones that are potential regulators of food anticipatory activities (FAAs) and responses that antecede food presentation. A number of circulating metabolic and hormonal factors originating outside the central nervous system (CNS), and modified by the timing of food intake are known, including corticosterone, insulin, ghrelin, leptin, adiponectin, free fatty acids, glucagon, glucocorticoids, thyroid hormones, etc. (reviewed in references^{8,23,25}). These systemically circulating signals can provide temporal information to tissues that bear appropriate receptors and thereby mediate the behavioral and physiological responses that are seen in anticipation of regularly timed meals.

Several criteria can be used to identify food-entrained cells and organs.²⁶ These include the requirement that FEOs and their output signal(s) must (i) antecede mealtime; (ii) stimulate activity in the absence of food deprivation; (iii) promote eating behavior; and (iv) elimination of the FEO output signal or its receptor should eliminate or attenuate FAAs. The FEOs that produce this putative signal should be (v) under circadian control; (vi) rhythmic in constant photic and (vii) nutrient conditions; and (viii) be entrained to the timing of food presentation. The presence of known clock genes/proteins is not a defining requirement for FEOs, though some cells may have such clock components. Here we consider three exemplars of glands that produce systemically circulating signals, the adrenal, pancreas, and stomach, recognizing that though none may be necessary for FAAs, each can provide temporal information for food-anticipatory responses.

Adrenal gland

The zona fasciculata of the human adrenal gland, situated between the glomerulosa and reticularis layers, is the site of the production of glucocorticoids. The circadian rhythm in adrenal production of steroids and the phase-shifting effects of feeding time on plasma corticoids have been thoroughly analyzed (Fig. 1).^{6,27–29} The circadian rhythm of corticoid secretion is under control of the SCN. Glucocorticoid receptors are widely expressed in peripheral tissues and corticoids can thereby act widely as input signals to oscillators.^{30–32} Adrenal hormones, however, while sufficient to entrain steroid receptor-containing cells, are not necessary for food-entrained behavior as adrenalectomized animals and those deficient in the glucocorticoid receptor continue to express FAA.^{7,30,33–35} There is evidence, however, for a physiological role of adrenal hormones.³⁶ Phase resetting in liver and kidney peripheral oscillators is slow when feeding time is changed from night to day and faster when changed from day to night. The resetting response is hastened in tissues from adrenalectomized animals suggesting that glucocorticoids prevent the uncoupling of

peripheral and central circadian oscillators that occurs with restricted feeding times. The important conclusion is that the inertia in the resetting of circadian gene expression in liver and kidney is not an intrinsic property of peripheral oscillators, but is caused by glucocorticoids. This is one example of a response that emerges from the interactions among oscillators and their output signals.

Another emergent timing response is seen in an interesting study demonstrating the effects of duration of food access on corticosterone secretion (Fig. 1). Corticosterone levels were examined in rats kept under restricted daily meals of 0.5, 2, or 6 h each day (Fig. 1 in reference³⁷). With 0.5-h meal duration, adrenal steroid rhythms were elevated and the daily trough disappeared. Following a 2 h meal, a peak was seen prior to meal presentation time. After a 6-h meal, corticosterone peaked at the end of the food availability period, rather than in anticipation of meal time. This reveals a novel principle: the precise timing and duration of the meal have substantially different effects on temporal parameters of adrenal secretion, and the duration and timing of adrenal hormones could act to provide temporal cues.

In summary, the adrenal steroids meet the criteria suggested above for FEOs. Adrenal corticoid secretions (i) antecede mealtime; (ii) stimulate activity; (iii) promote eating behavior; and (iv) elimination of adrenal hormones or their receptors attenuate FAAs. The FEOs that produce adrenal secretions are (v) under circadian control; (vi) rhythmic in constant photic and (vii) ad lib nutrient conditions; and (viii) are entrained to the timing of food presentation. There are some indications that the adrenal hormones may affect eating behavior as corticosterone is produced in response to stress, and its levels can be abnormal in some models of obesity.^{38,39} Furthermore, stress may augment appetite in humans.⁴⁰

Pancreas

Insulin is synthesized by the β -cells of the pancreatic islets of Langerhans with extensive effects on glucose uptake and glycogen storage. There is a circadian rhythm in insulin secretion, detectable when glucose levels are clamped.⁴¹ Glucose is more effective than fat in phase-shifting the food-entrained circadian clock, consistent with a possible role of insulin.⁴² There are significant relationships between insulin and the core circadian clock gene machinery. Real-time reverse transcription–polymerase chain reaction (RT-PCR) in rat pancreatic tissue indicates a robust circadian expression of clock genes *Per1* and *Bmal1*, suggesting the existence of pancreatic oscillators.⁴³ A genome-wide small-interfering RNA screen in a cellular clock model indicates that over a dozen components of the insulin pathway are transcriptionally regulated by the circadian clock.⁴⁴ Down-regulation of several distinct components of the insulin pathway results in either the lengthening or the shortening of circadian period.

Just as was shown for adrenal steroids (Fig. 1³⁷), there is an effect of duration of fasting on insulin levels. Starvation resulted in a progressive decrease of insulin secretion in response to clamped glucose levels (15 mM).⁴⁵ The CNS has insulin receptors, and thus insulin could serve as a signal for feeding.^{46,47} Under normal conditions, where glucose levels are not clamped, a premeal insulin peak coincides with the premeal elevation of plasma corticosterone and an increase in body temperature^{48–51} and this functions to minimize prandial increases of blood glucose.^{52,53} In contrast to ghrelin (see From Cells and Glands to Behaviours below) insulin does not stimulate the appetitive component of FAAs and does not augment locomotor activity during food deprivation.⁵⁴ Peripherally administered insulin increases food intake (through glucose decrease), while centrally administered insulin decreases food intake.⁵⁵

Taken together, the foregoing evidence indicates that insulin secreting cells meet many of the criteria required of FEOs and suggest that insulin could signal regularly scheduled food

availability. Pancreatic insulin secretion antecedes mealtime and elimination of insulin signaling alters FAAs (see section 3 below). The FEOs that produce insulin secretion are under circadian control, and are entrained to the timing of food presentation.

Stomach and gastrointestinal tract

The epithelium of the stomach forms deep pits which are the loci of numerous glands that secrete digestive hormones. Of interest here is the hormone ghrelin, produced mainly by oxyntic cells lining the corpus and antrum of the human stomach (Fig. 2). Plasma levels of ghrelin fluctuate diurnally, with a peak in the day and a trough at night.⁵⁶ Additionally, plasma levels of ghrelin increase before mealtime and decrease after meals.^{57–59} Ghrelin is a 28-amino acid peptide, and an endogenous ligand for growth-hormone secretagogue receptor. The numerous E-box elements, known targets of circadian clock proteins present in the promoter region of the ghrelin gene, indicate that ghrelin is likely a clock-controlled gene.⁶⁰ Of the known gastrointestinal tract hormones, many decrease food intake, while ghrelin alone stimulates food intake.⁶¹ Exogenous ghrelin administered systemically or in a number of brain sites stimulates eating, even following satiation.^{26,62} This makes ghrelin-secreting oxyntic cells of the stomach attractive candidates in the feeding timing system.

We have demonstrated that ghrelin-secreting oxyntic cells of the stomach are oscillators entrained by food and that they produce a systemic output signal that could set the phase of other oscillators in the body.²⁶ Oxyntic cells express both ghrelin and the circadian clock proteins PER1 and PER2 (Fig. 2). The expression of PER1, PER2, and ghrelin is rhythmic in light:dark (LD) cycles, in constant darkness with ad libitum food access, and after a period of total food deprivation. Clock mutant *mPer1,mPer2* double-mutant mice lack rhythmic locomotor behavior, and also lack a pre-meal decrease in oxyntic-cell ghrelin. The phase of rhythmic ghrelin and PER expression are set by prior feeding time, rather than by the LD schedule. The results indicate that oxyntic gland cells of the stomach contain canonical circadian clock genes that are entrained by food, and produce a timed ghrelin output signal.

Ghrelin receptors are widely distributed in both brain and peripheral sites, and ghrelin injection directly into any one of the brain sites stimulates eating behavior in sated animals.²⁶ It is interesting to note that the SCN too responds to direct ghrelin application.⁶³ Ghrelin applied *in vitro* to cultured SCN slices induces an approximately 3-h phase advance in electrical activity at circadian time 6. Ghrelin also phase-advances the rhythm of PER2::LUC (Period2::Luciferase) expression in cultured SCN explants from *mPer2* (Luc) transgenic mice. In the same report, *in vivo*, intraperitoneal administration of a synthetic ghrelin analog, growth hormone-releasing protein-6 (GHRP-6), failed to alter the circadian phase in ad libitum-fed animals, but induced a phase advance when injected after 30 h of food deprivation. These results indicate that ghrelin exerts a direct action on the SCN and the system as a whole appears to become sensitive to ghrelin following food restriction. Like corticoids and insulin, ghrelin can also provide a signal of duration of food deprivation. Comparing fasting of 12 *versus* 17 h duration, there was a significant increase in plasma ghrelin and hunger rating in healthy humans.⁶⁴

The foregoing data indicate that ghrelin-secreting cells are FEOs. The gastrointestinal tract has its own nervous system, the enteric nervous system, and under normal conditions the activities of this nervous system are coordinated with those of the CNS. It is capable, however, of maintaining motor and sensory function independent of nervous input from the CNS. In addition, specialized enteroendocrine cells of the gastrointestinal system secrete numerous meal-associated hormones that are transmitted to the brain neurally via vagal afferents, or humorally as circulating ligands for specific receptor populations in the CNS and periphery. Many of the hormones found in the gastrointestinal tract are also produced in

the CNS,^{61,65} indicating that either source of the signal can serve as a source of eating-related signals.

In summary, ghrelin-secreting cells meet all the criteria required of FEOs. Ghrelin secretion (i) antecedes mealtime; (ii) stimulates locomotor activity in the absence of food; (iii) promotes eating behavior; and (iv) elimination of the ghrelin receptor in knockout animals alters the timing of FAAs, but does not eliminate anticipatory behavior (Fig. 3; discussed further below). The oxyntic cells that produce insulin secretions are (v) under circadian control; (vi) rhythmic in constant photic and (vii) ad lib nutrient conditions; and (viii) are entrained to the timing of food presentation. Ghrelin, however, is not required for the expression of FAAs.

INTERACTIONS AMONG HORMONES

Not surprisingly, there are important interrelationships among the major endocrine hormones. These contribute to feedback loops that modulate the circadian timing system. Here we consider the relationships among corticoids, melatonin, insulin, and glucagon.

Corticoid–melatonin interactions

Mt1 melatonin receptors occur in the adrenal gland, and melatonin inhibits adrenocorticotrophic hormone (ACTH)-stimulated cortisol production in capuchin monkeys.⁶⁶ Melatonin directly inhibits the expression of clock genes in adrenocortical explants.⁶⁷ Under restricted feeding conditions, there is a bimodal peak of adrenal hormones, one related to feeding time and the other to the LD cycle.³⁶ Thus, the adrenal gland is an important site of integration of photic and feed/fasting signals.^{27,68} It receives light-derived neural input from the SCN, possibly decoded by melatonin signals, and neuroendocrine signals via ACTH, each of which influence circadian timekeeping.

Insulin–glucagon links

Insulin and glucose levels are temporally linked. Following a meal, the amount of insulin secreted into the blood increases as the blood glucose rises, and in parallel, during fasting, as blood glucose falls, insulin secretion decreases. Glucose levels are regulated by glucagon, which is produced by the pancreatic alpha cells when blood glucose is low. Centrally, glucagon delivered intracerebroventricularly depresses food intake.⁶⁹ The concentration of glucagon is decreased during FAA compared to its concentration prior to FAA in both intact and SCN-lesioned subjects, and this difference is not observed in control, equally fasted rats that have not been previously entrained to a daily meal.⁷⁰ Thus, a drop in glucose (regulated by glucagon) or insulin could predict the initiation of a meal and trigger FAAs, as both are influenced by the duration of fasting.

Insulin–glucocorticoid links

Further evidence of functional interrelationships between insulin, circadian clocks, and other systemically circulating signals are seen in the interaction of insulin, glucocorticoids, and leptin with the circadian system. Per2Brdm1 mutant mice treated with dexamethasone (a glucocorticoid analog) are more sensitive to insulin compared with dexamethasone-treated controls, as they have a more substantial decrease in blood sugar in response to the insulin than do their wild-type counterparts.^{71,72} Elevated leptin levels improve insulin sensitivity and glucose tolerance^{73,74} and leptin resistance is associated with obesity and diabetes. Leptin itself is under circadian control and cycles in antiphase to corticoid levels.^{72,75}

Melatonin–insulin links

The pineal hormone melatonin is under circadian control through the SCN and is not shifted by temporally restricted food access.³³ Photic input from the SCN reaches the pineal gland via a multisynaptic route. The secretion of melatonin occurs during the dark phase of the LD cycle, thereby encoding night duration. The best known function of melatonin is its role in the regulation of seasonal reproductive cycles (reviewed in reference⁷⁶). Another function of melatonin is its role as a zeitgeber in the rat pancreatic β -cell, which has a circadian insulin secretion pattern in isolated pancreatic islets.⁷⁷ Both phase shifts and amplitude of circadian responses are altered in melatonin receptor-knockout mice with respect to the clock gene- or clock-output transcripts PER1, DBP, and RevErb α in the pancreas and liver. Insulin secretion from isolated islets of melatonin receptor MT1, MT2 or MT1 and MT2 double melatonin receptor-knockout animals was increased relative to the wild type. These data support the idea that melatonin can set the phase of major organs involved in blood glucose regulation and negatively acts on insulin secretion.

FROM CELLS AND GLANDS TO BEHAVIORS AS READOUTS OF ANTICIPATORY RESPONSES

When food is available *ad libitum*, the time of meals coincides with the active portion of the daily sleep/rest cycle. In these conditions, the component of the behavior under control of the SCN cannot be distinguished from that under the control of metabolic factors related to food availability. However, when food availability is temporally restricted to the inactive part of the day, periods of activity are split into two bouts, one determined by the SCN and the other by the anticipation of food. Importantly, comparison of the temporal distribution of responses under *ad libitum* *versus* restricted feeding conditions suggests that the SCN and food-related components are additive. An example selected from many such instances in the literature is shown in Figure 4.⁷⁸ Here it can be seen that under *ad lib* feeding conditions, the mouse restricts its activity to the dark component of the day–night cycle. When food is restricted to the light phase (ZT6–10), the animal keeps the total amount of activity constant, but increases activity in the day and reduces activity at night. It is likely that the coherent bout of activity seen under *ad libitum* conditions is actually made up of two separate components, distinguishable only under specific environmental conditions.

The evidence suggests that in the presence of a normally functioning SCN, the major known orexigenic (appetite stimulating) and anorexigenic (appetite inhibiting) hormones can affect the amplitude, phase, and period of anticipatory responses. In some cases, the anticipatory responses are enhanced, while in others, anticipatory behaviors are diminished. In the case of abnormal or absent SCN, control of circadian activity rhythms is shifted and is relegated entirely to the food-derived cues.

Reduction of food anticipatory activity and loss of orexigenic hormone effects

Elimination of each of the most potent orexigenic hormones and neurohormones result in reduced FAAs. As noted above, ghrelin is an orexigenic hormone, secreted in anticipation of regularly scheduled meals. Exogenous ghrelin administration increases locomotor activity even in the absence of food (appetitive response) and stimulates eating behavior (consummatory response) even in nondeprived animals^{26,79}. There are several differences between wild type and those lacking ghrelin receptors.^{26,80} In the latter, the daily anticipatory activity bout is delayed, and begins closer to the time of food availability compared to wild-type animals (Fig. 3). Nevertheless, both groups produce the same rate of wheel revolutions/10 min bin at the time of peak activity, and there is no difference in total amount of daily activity between wild-type and ghrelin receptor-knockout mice during days of food restriction. There is, however, a significant difference in how their activity is

partitioned. The inter-group differences lie in the amount of activity under control of food *versus* the SCN,²⁶ with the ghrelin receptor-knockout animal showing less food-related activity. For individuals in both groups, once an animal begins its daily anticipatory bout, it keeps running until the usual time of food availability, as though a switch has changed its state or a threshold has been reached (Fig. 3).²⁶

Enhanced food anticipatory activity and anorexigenic stimuli

Insulin—The diabetic genetically obese Zucker rats show enhanced food-anticipatory responses compared to their lean counterparts.⁸¹ (Zucker rats, bearing a mutation in the leptin receptor are a widely used genetic model for obesity research. The lean Zucker rat bears the dominant trait [Fa/Fa] or [Fa/fa], while the obese Zucker rat bears a recessive trait [fa/fa]). They start their daily food-bin oriented activity earlier than do the lean rats and they also reach a higher peak of activity in anticipation of food (Fig. 5). The nighttime component of food-bin related activity does not differ between groups.

The diabetic db/db mouse has a point mutation in the gene for the leptin receptor. In the db/db mouse, SCN expression of clock genes is not altered, while locomotor activity and liver clock gene expression are attenuated under ad lib feeding conditions.⁸² Nighttime restricted feeding leads to a recovery from the diminished locomotor activity and altered oscillation of peripheral clocks and *mPai-1* mRNA rhythm. Thus, it appears that attenuation and arrhythmicity of the locomotor activity and clock gene mRNA rhythm in the liver does not result from altered SCN oscillator function, but rather from abnormalities in the output mechanism mediating locomotor activity and peripheral clock resetting. The obese Zucker (*fa/fa*) rats showed reduced circadian amplitude of body temperature and activity, though with a period similar to that of lean control mice.⁸³ In contrast, the *db/db* mice had a much greater attenuation of locomotor activity rhythm and loss of rhythmicity, perhaps related to their more severe diabetic symptoms.

Orexin—Genetically orexin-neuron ablated mice showed decreased amounts of anticipatory activity but started their daily activity bout at about the same time as controls during restricted feeding.^{78,84} There were no differences between groups in amount of daily running nor in amount of food intake. As seen in the case of ghrelin-receptor knockout animals, the differences between orexin-ablated and control animals were in the allocation of activity to food-related *versus* SCN-driven components.

Food anticipatory activity and altered SCN function—FAAs have been examined in several conditions in which SCN function is abnormal. For example, in constant darkness, CS mice (an inbred strain established from hybrids between the NBC and SII strain) show unstable free-running periods and bimodal daily activity patterns. Control mice tested in a restricted feeding paradigm free-run from the phase set by the SCN pacemaker when released into ad lib conditions in constant darkness (DD). In contrast, in CS mice, the free-running component of circadian behavioral rhythms entrains to restricted feeding under constant darkness.^{85,86}

VIP—/—Another example of circadian rhythmicity in the absence of an effective SCN master clock can be seen in the case of mutant mice lacking VIP receptors (*Vip2r*^{-/-} mice). In LD cycles both wild-type and *Vip2r*^{-/-} mice displayed locomotor activity at the onset of darkness and in anticipation of food, presumably an effect of masking by light. In constant darkness, *Vip2r*^{-/-} mice displayed only a single daily bout of activity in anticipation of food, while their wild-type counterparts partition their activity into two components – timed to dark onset and to food availability.⁸⁷ The activity component that is normally regulated by

the SCN is absent in these mutant animals, while the food-regulated component is augmented.

The mechanistic basis of anticipatory timing of food is not well understood. While circadian locomotor rhythmicity is disrupted in mutant animals lacking normal core clock genes, such animals continue to display timed food anticipatory behaviors (but see reference⁸⁸). This was first demonstrated in NPAS knockout animals and subsequently reported in a variety of other clock mutant animals.^{89–92} One possibility is that FAA responses involve more than a circadian timing system, a topic we consider below. Another possibility is that the necessary “core clock genes” differ for FAA and for other controlled circadian rhythms. The recent discovery of a molecular mechanism through which metabolic cycles interact with the circadian clock network may be helpful in resolving these questions. Here, it has been proposed that adenosine monophosphate–activated protein kinase (AMPK), an enzyme that responds to nutrient availability, directly phosphorylates the clock protein cryptochrome 1 (CRY1), thus preparing it for degradation.⁹³

Summary—The foregoing data indicate that the timing of food anticipatory activity can be altered in many different ways. Signals that affect feeding behavior modulate the precision of anticipatory timing in the sense that their behavior is more closely timed to the onset of food either increasing (e.g. ghrelin receptor-knockout animals²⁶) or decreasing precision (e.g. leptin receptor-mutant Zucker obese rats⁸¹). Other mutations shift the amount of behavior that is under the control of SCN-related *versus* food-derived cues (CS mice and orexin-ablated animals). The results at the behavioral level provoke the question of how these orexigenic and anorexigenic transmitters and hormones provide cues that can affect the timing of anticipation of food. As this selective review suggests, light- and food-entrained output signals each normally contribute to the timing of activity. In the presence of conflicting information from food and photic cues, the independent contribution of these components is discernible. In this view, the daily rest/activity cycles are a composite of the influences exerted by both of these mechanisms. The implication is that each component may be regulated by some common or separate time cues. In order to explore the possibility of separate timing mechanisms for SCN and metabolic control, the two components must be separated and evaluated independently. Also, it is not sufficient to show that each component is under circadian control: it is also necessary to test whether there is a contribution of a homeostatic component. The ways in which multiple signals can interact is next explored.

INTERACTIONS AMONG TEMPORAL SIGNALS FOR ANTICIPATORY BEHAVIORS

It is clear that many interacting signals, arising from both central and peripheral oscillators, contribute to FAAs. Because much of the control involves circadian timing, it is convenient to think of the signal as being produced by an oscillator with the appropriate period. Though we conceptualize the underlying circadian signal as sinusoidal, the actual behavior we observe has the character of a square wave. An examination of most published actograms shows a fairly abrupt transition from inactivity to activity as the time of food availability draws near. One way of exposing this feature is to plot the cumulative activity within a day as was done for individual subjects in LeSauter *et al.*²⁶ (Fig. 3). A straight line in such a plot indicates a constant rate of activity. These plots indicate that all subjects show a rapid transition to a relatively steady rate of anticipatory activity. In this study,²⁶ the difference between the ghrelin receptor-knockout and wild-type animals appears to be in the start time for their anticipatory activity and not in the rate at which it occurs once it begins. At a conceptual level, the square wave character of the anticipatory behavior arises if we assume

that the anticipatory behavior begins when the circadian signal exceeds some threshold, and ends when food is presented or when the signal falls below that threshold level. When conceptualized in this way we can specify the ways that various timing signals from oscillators might interact with one another to produce the observed anticipatory behavior, shown schematically in Figure 6.

First, consider the case where two oscillators are in fixed phase with one another and the signals add together. If the oscillators were perfectly in phase, the composite signal would cross the threshold sooner and reach higher levels than would a signal that lacked either component. Zucker obese rats show this pattern in comparison to their Zucker lean controls (Fig. 5, left panel⁸¹). If FAAs are the result of a composite signal, and if one of the sources of temporal information is removed, then the signal would cross the threshold later and reach lower levels at the time of food availability. Such a response is seen in the ghrelin receptor-knockout mice²⁶ and orexin-deficient mice⁷⁸ (Fig. 5, right panel) which both show the later onset and lower level of FAA compared to their respective controls. Second, timing signals may interact in other ways to produce this same observed effect on the onset and amplitude of FAA. For example, if one signal modulates the amplitude of the stimuli that directly lead to behavioral output, onset times will be earlier the greater the amplitude. A third way that changes in the onset and amplitude of the anticipatory activity could be realized is if one signal advances or delays other signals that control the behavioral output. If there is a phase delay of the signal that triggers activity then the response will begin later and consequently may not reach as high a level at meal onset. Yet a fourth mechanism that would produce a shift in FAA onset is if the elimination of one timing signal changed the period of the remaining oscillators. If the period were lengthened, then meals would begin at an earlier phase, again producing a delayed onset and lower level of anticipation prior to a meal.

A final possibility is that altering the number of sources of temporal information changes the variability or precision of timing in addition to changing the time of onset and maximum level of anticipation. An increase in variability of FAAs is seen when lighting conditions are changed from LD to DD,⁹¹ thereby removing dawn and dusk as sources of temporal information. An empirical example of this phenomenon is seen in Figure 7. Also, the precision of the FAA will reflect the precision of the underlying timers. For example, if two sources of temporal information have independent sources of variability the additive composite signal would have greater variability than individual sources. If this were the case, then paradoxically, removing a source of temporal information might actually increase the precision of the time signal (reduce its variability). Changes in the variability of onset times are reflected in the slope of the average function that tracks increases in FAA over time. If eliminating a source of information reduced variability then the function would become steeper. Storch and Weiss⁹² found that BMAL knockout mice showed a steeper anticipation function than wild type, consistent with the idea that the mutants' anticipatory response is timed more precisely. However, the data are presented as averaged across subjects, indicating reduced between-subject variability. Thus we cannot tell if the within-individual variability is also lower, as would be expected with increases in timing precision.

The consideration of how performance might differ in LD and DD highlights other potential sources of temporal information regulating the start time of FAAs. While dawn and dusk cues entrain circadian responses, they also provide additional sources of timing information, thereby increasing the precision of the anticipatory response. In LD, available time cues include lights on, lights off, food on, food off, and all of the endogenous oscillators entrained by light or food. Consistent with the perspective that multiple sources of temporal information are used to regulate behavior,⁹⁴ trained animals searched three different locations at three different times of day. When light-to-dark transitions were eliminated on a

test day and one of the meals omitted, the rats visited correct locations with impaired performance indicating that timing of the intervals between light transitions and meals and/or the time between meals contributed to accurate performance. However, they continued to visit correct locations at above-chance levels near the correct time even when the prior meal was omitted indicating that the circadian systems also contributed to the correct anticipation of where a meal would be found at a particular time.

Additional direct evidence that multiple sources of temporal information are integrated in the regulation of anticipatory behavior comes from a lovely series of experiments in pigeons.⁹⁵ The results demonstrate that animals simultaneously use whatever temporal cues are available. In this study, animals were housed in LD 14:10 (on 06.00; off 20.00 hours). Three times each day, an illuminated key light cue (of 60 min duration) signaled that food would be available for a 30-min meal interval, starting 30 min after key light onset. That is, a breakfast, lunch, and dinner meal opportunity, signaled by the cue-light became available at 07.00, 12.00, and 17.00 hours. To get the food, the pigeon had to peck at a key, providing the experimenter with the opportunity to measure its estimation of time. For all three cued meals, the pigeon increased its pecking behavior as the time of food availability approached. In the experimental days of interest, the dawn, the cue-light, or both the dawn and cue-light were either advanced or delayed by 1 h and meals omitted. If the interval from the onset of dawn and/or cue-light controls the timing, then the peak should be shifted by 1 h. If the circadian system controls anticipation, then the time of peak responding should remain unchanged. The results show that peak responding occurred later than usual but was not shifted by the full hour. The peak was a compromise between the circadian and interval information about the time of food availability. In additional conditions studied, peak responding occurred at an earlier time of day when cues were advanced that was intermediate between that predicted by either exclusive circadian or interval control of anticipation. Thus it appears that all of the sources of temporal information available to animals about the time of food availability are used to regulate anticipatory activity.

In the last two examples animals were presumably using mechanisms that allowed them to time arbitrary intervals such as between dawn and meal availability or the time between successive meals. The timing of arbitrary intervals in non-circadian ranges has been extensively studied in experimental psychology (see reference⁹⁶ for a review of how interval timing regulates food anticipation). A ubiquitous characteristic of interval timing is that the variability of timing increases with the duration of the interval being timed. Further, this variability is substantially greater than that observed in circadian systems.⁹⁵ Thus if interval timing contributes to FAAs in standard paradigms, animals should become more variable as the duration of the interval being timed increases. Stephan and Becker⁹⁷ varied the meal duration and thus manipulated the time from the end of a meal until the next one became available. The time between meals ranged from 12 to 20 h. The average increase in anticipation prior to a meal (their Fig. 4) began earlier and had a shallower slope at the longer intervals. This is consistent with the idea that the timing of the intermeal interval also contributes to FAA in standard paradigms. Because only average data are presented in FAA studies, we cannot determine if the conditions generated more variation between individuals or if variation in individual subjects' timing precision caused the changed character of the average functions.

One way in which the timing behavior of individual animals can be examined is by using a modification of the procedures first described by Yoshihara et al.⁹⁸ Here rats were trained on a restricted feeding paradigm. Then they were tested repeatedly in a food deprivation condition after 7–10 days of ad lib feeding (Fig. 8). In that study the goal was to determine how long the memory for the restricted feeding would be retained – as measured by locomotor activity. This design could be modified to test animals for various intervals of

food deprivation, and ask whether variability of the response in the deprivation condition was proportional to the duration of food deprivation. If this measure has the scalar property, this would be strong evidence for the contribution of interval timing.

SUMMARY: FROM SYSTEMS TO CELLS TO TIMING SIGNALS

The foregoing data indicates that signals that affect feeding behavior modulate anticipatory timing. The implication is that at the level of behavior each component of FAA- and SCN-controlled activity may be regulated by some common and some separate time cues. In order to explore the timing mechanisms for SCN and metabolic control, the food- and SCN-related components must be separated and evaluated independently.

In the natural environment a plethora of cues including endogenous oscillators, dawn and dusk, and other environmental cues can provide information that signal when specific food types might be available at specific locations. These include endogenously occurring signals such as those reviewed here, as well as arbitrary signals learned through experience. At the behavioral level, reliable indicators of food availability eventually control food intake. Animals can learn to make meal-anticipatory behaviors in response to arbitrary stimuli that reliably indicate food availability, and those same stimuli can elicit eating, even in sated animals.⁹⁹ Consequently, changes in behavior and physiology in anticipation of predictable meals are the result of a composite signal arising from central and peripheral changes that are entrained to times of food availability.

Numerous internal and external cues have the potential to regulate the timing of the physiological and behavioral responses involved in food anticipation. We concur with Woods,¹⁰⁰ who suggests that “when to eat is largely dictated by environmental factors, especially time of day ... In a predictable world, individuals learn to utilize signals farther and farther from actual energy content.” Individuals differ in their sensitivity to internal and external signals which vary over circadian time, time since the previous meal, time until the next meal, or after food deprivation. All of these cues are gated to circuits controlling behavior. Individuals detect changes in internal and external parameters that occur as a consequence of the brain and body preparing for an impending meal and interpret those changes as “hunger.”

The brain is a key participant in the control of food intake. The brain, among other organs, integrates information from the body on energy status and influences the entry of nutrients into blood and tissues. It is unclear whether or not a master food entrainable clock, homologous to the light-entrainable clock, normally synchronizes the phase of various FEOs. Whether there exists a master synchronizer, or factious sets of such masters in the brain at the systems level, the food-driven circadian timekeeping system consists of interacting brain structures and peripheral organs that interact to form feedback loops and results in a 24-h oscillation.^{10,23,101} The present review highlights the value of exploring separately the contributions to timing of the light-entrainable oscillators of the SCN and the FEOs of extra-SCN central and peripheral systems.

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References

1. Moore RY, Lenn NJ. A retinohypothalamic projection in the rat. *J Comp Neurol.* 1972; 146:1–14. [PubMed: 4116104]
2. Stephan FK, Zucker I. Circadian rhythms in drinking behavior and locomotor activity are eliminated by suprachiasmatic lesions. *Proc Natl Acad Sci USA.* 1972; 54:1521–7.
3. Moore RY, Eichler VB. Loss of circadian adrenal corticosterone rhythm following suprachiasmatic nucleus lesion in the rat. *J Comp Neurol.* 1972; 146:1–14. [PubMed: 4116104]
4. Richter CP. A behavioristic study of the activity of the rat. *Comp Psychol Monog.* 1922; 1:56.
5. Krieger DT, Hauser H, Krey LC. Suprachiasmatic nucleus lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. *Science.* 1977; 193:398–9. [PubMed: 877566]
6. Krieger DT. Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology.* 1974; 95:1195–201. [PubMed: 4426285]
7. Boulous Z, Rosenwasser AM, Terman M. Feeding schedules and the circadian organization of behavior in the rat. *Behav Brain Res.* 1980; 1:39–65. [PubMed: 7284080]
8. Stephan FK. The “other” circadian system: food as a Zeitgeber. *J Biol Rhythms.* 2002; 17:284–92. [PubMed: 12164245]
9. Davidson AJ. Lesion studies targeting food-anticipatory activity. *Eur J Neurosci.* 2009; 30:1658–64. [PubMed: 19863659]
10. Escobar C, Cailotto C, Angeles-Castellanos M, Delgado RS, Buijs RM. Peripheral oscillators: the driving force for food-anticipatory activity. *Eur J Neurosci.* 2009; 30:1665–75. [PubMed: 19878276]
11. Antle MC, Silver R. Neural basis of timing and anticipatory behaviors. *Eur J Neurosci.* 2009; 30:1643–9. [PubMed: 19878281]
12. Sun ZS, Albrecht U, Zhuchenko O, Bailey J, Eichele G, Lee CC. RIGUI, a putative mammalian ortholog of the *Drosophila* period gene. *Cell.* 1997; 90:1003–11. [PubMed: 9323128]
13. Tei H, Okamura H, Shigeyoshi Y, et al. Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. *Nature.* 1997; 389:512–16. [PubMed: 9333243]
14. Balsalobre A, Damiola F, Schibler U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell.* 1998; 93:929–37. [PubMed: 9635423]
15. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 2000; 14:2950–61. [PubMed: 11114885]
16. Davidson AJ, Poole AS, Yamazaki S, Menaker M. Is the food-entrainable circadian oscillator in the digestive system? *Genes Brain Behav.* 2003; 2:32–9. [PubMed: 12882317]
17. Stokkan KA, Yamazaki S, Tei H, Sakaki Y, Menaker M. Entrainment of the circadian clock in the liver by feeding. *Science.* 2001; 291:490–3. [PubMed: 11161204]
18. Schibler U, Ripperger J, Brown SA. Peripheral circadian oscillators in mammals: time and food. *J Biol Rhythms.* 2003; 18:250–60. [PubMed: 12828282]
19. Butler MP, Silver R. Basis of robustness and resilience in the suprachiasmatic nucleus: individual neurons form nodes in circuits that cycle daily. *J Biol Rhythms.* 2009; 24:340–52. [PubMed: 19755580]
20. Liu AC, Welsh DK, Ko CH, et al. Intercellular coupling confers robustness against mutations in the SCN circadian clock network. *Cell.* 2007; 129:605–16. [PubMed: 17482552]
21. Hastings MH, Reddy AB, Maywood ES. A clockwork web: circadian timing in brain and periphery, in health and disease. *Nat Rev Neurosci.* 2003; 4:649–61. [PubMed: 12894240]
22. Verwey M, Amir S. Food-entrainable circadian oscillators in the brain. *Eur J Neurosci.* 2009; 30:1650–7. [PubMed: 19863660]
23. Mendoza J. Circadian clocks: setting time by food. *J Neuroendocrinol.* 2007; 19:127–37. [PubMed: 17214875]
24. Guo H, Brewer JM, Champhekar A, Harris RB, Bittman EL. Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. *Proc Natl Acad Sci USA.* 2005; 102:3111–16. [PubMed: 15710878]

25. Froy O. The relationship between nutrition and circadian rhythms in mammals. *Front Neuroendocrinol.* 2007; 28:61–71. [PubMed: 17451793]
26. LeSauter J, Hoque N, Weintraub M, Pfaff DW, Silver R. Stomach ghrelin-secreting cells as food-entrainable circadian clocks. *Proc Natl Acad Sci USA.* 2009; 106:13582–7. [PubMed: 19633195]
27. Dickmeis T. Glucocorticoids and the circadian clock. *J Endocrinol.* 2009; 200:3–22. [PubMed: 18971218]
28. Shen L, Carey K, Wang DQ, Woods SC, Liu M. Food-entrained rhythmic expression of apolipoprotein E expression in the hypothalamus of rats. *Brain Res.* 2009; 1273:66–71. [PubMed: 19362542]
29. Unger F, Halberg F. Circadian rhythm in the in vitro response of mouse adrenal to adrenocorticotrophic hormone. *Science.* 1962; 137:1058–60. [PubMed: 13923797]
30. Balsalobre A, Brown SA, Marcacci L, et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science.* 2000; 289:2344–7. [PubMed: 11009419]
31. Rosenfeld P, Van Eekelen JA, Levine S, De Kloet ER. Ontogeny of the type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study. *Brain Res.* 1988; 470:119–27. [PubMed: 3409041]
32. Rosenfeld P, Van Eekelen JAM, Levine S, De Kloet ER. Ontogeny of Corticosteroid Receptors in the Brain. *Cell Mol Neurobiol.* 1993; 13:295–319. [PubMed: 8252605]
33. Boulos Z, Terman M. Food availability and daily biological rhythms. *Neurosci Biobehav Rev.* 1980; 4:119–31. [PubMed: 6106914]
34. Davidson AJ, Stephan FK. Feeding-entrained circadian rhythms in hypophysectomized rats with suprachiasmatic nucleus lesions. *Am J Physiol.* 1999; 277 (Pt 2):R1376–84. [PubMed: 10564210]
35. Stephan FK, Swann JM, Sisk CL. Anticipation of 24-hr feeding schedules in rats with lesions of the suprachiasmatic nucleus. *Behav Neural Biol.* 1979; 25:346–63. [PubMed: 464979]
36. Le Minh N, Damiola F, Tronche F, Schutz G, Schibler U. Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *EMBO J.* 2001; 20:7128–36. [PubMed: 11742989]
37. Honma KI, Honma S, Hiroshige T. Critical role of food amount for prefeeding corticosterone peak in rats. *Am J Physiol.* 1983; 245:R339–44. [PubMed: 6614204]
38. Rosenbaum M, Leibel RL, Hirsch J. Obesity. *N Engl J Med.* 1997; 337:396–407. [PubMed: 9241130]
39. Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. *Cell.* 1996; 87:377–89. [PubMed: 8898192]
40. Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology.* 2001; 26:37–49. [PubMed: 11070333]
41. Boden G, Ruiz J, Urbain JL, Chen X. Evidence for a circadian rhythm of insulin secretion. *Am J Physiol.* 1996; 271 (Pt 1):E246–52. [PubMed: 8770017]
42. Stephan FK, Davidson AJ. Glucose, but not fat, phase shifts the feeding-entrained circadian clock. *Physiol Behav.* 1998; 65:277–88. [PubMed: 9855477]
43. Muhlbauer E, Wolgast S, Finckh U, Peschke D, Peschke E. Indication of circadian oscillations in the rat pancreas. *FEBS Lett.* 2004; 564:91–6. [PubMed: 15094047]
44. Zhang EE, Liu AC, Hirota T, et al. A genome-wide RNAi screen for modifiers of the circadian clock in human cells. *Cell.* 2009; 139:199–210. [PubMed: 19765810]
45. Hahn HJ, Fiedler H. Investigations on isolated islets in vitro. IX. Influence of food deprivation and glucose refeeding in vitro on insulin secretion. *Acta Diabetol Lat.* 1974; 11:457–67. [PubMed: 4620122]
46. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr. Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev.* 1992; 13:387–414. [PubMed: 1425482]
47. Chandra R, Liddle RA. Neural and hormonal regulation of pancreatic secretion. *Curr Opin Gastroenterol.* 2009; 25:441–6. [PubMed: 19535978]

48. De Vries J, Strubbe JH, Wildering WC, Gorter JA, Prins AJ. Patterns of body temperature during feeding in rats under varying ambient temperatures. *Physiol Behav.* 1993; 53:229–35. [PubMed: 8446685]
49. Even PC, Nicolaidis S. Adaptive changes in energy expenditure during mild and severe feed restriction in the rat. *Br J Nutr.* 1993; 70:421–31. [PubMed: 8260469]
50. Powley TL, Berthoud HR. Diet and cephalic phase insulin responses. *Am J Clin Nutr.* 1985; 42 (Suppl):991–1002. [PubMed: 3933326]
51. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes.* 1981; 30:219–25. [PubMed: 7009270]
52. Berthoud HR, Bereiter DA, Trimble ER, Siegel EG, Jeanrenaud B. Cephalic phase, reflex insulin secretion. Neuroanatomical and physiological characterization. *Diabetologia.* 1981; 20 (Suppl): 393–401. [PubMed: 7014335]
53. Woods SC. The eating paradox: how we tolerate food. *Psychol Rev.* 1991; 98:488–505. [PubMed: 1961770]
54. Campbell BA, Fibiger HC. Effects of insulin on spontaneous activity during food deprivation. *J Comp Physiol Psychol.* 1970; 71:341–6. [PubMed: 5480866]
55. Woods SC, Lutz TA, Geary N, Langhans W. Pancreatic signals controlling food intake; insulin, glucagon and amylin. *Philos Trans R Soc Lond B Biol Sci.* 2006; 361:1219–35. [PubMed: 16815800]
56. Sanchez J, Oliver P, Pico C, Palou A. Diurnal rhythms of leptin and ghrelin in the systemic circulation and in the gastric mucosa are related to food intake in rats. *Pflugers Arch.* 2004; 448:500–6. [PubMed: 15107997]
57. Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, Woods SC. Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology.* 2006; 147:23–30. [PubMed: 16179409]
58. Frecka JM, Mattes RD. Possible entrainment of ghrelin to habitual meal patterns in humans. *Am J Physiol Gastrointest Liver Physiol.* 2008; 294:G699–707. [PubMed: 18187517]
59. Sugino T, Hasegawa Y, Kurose Y, Kojima M, Kangawa K, Terashima Y. Effects of ghrelin on food intake and neuroendocrine function in sheep. *Anim Reprod Sci.* 2004; 82–83:183–94.
60. Kanamoto N, Akamizu T, Tagami T, et al. Genomic structure and characterization of the 5'-flanking region of the human ghrelin gene. *Endocrinology.* 2004; 145:4144–53. [PubMed: 15142980]
61. Strader AD, Woods SC. Gastrointestinal hormones and food intake. *Gastroenterology.* 2005; 128:175–91. [PubMed: 15633135]
62. Ueno H, Yamaguchi H, Kangawa K, Nakazato M. Ghrelin: a gastric peptide that regulates food intake and energy homeostasis. *Regul Pept.* 2005; 126:11–19. [PubMed: 15620408]
63. Yannielli PC, Molyneux PC, Harrington ME, Golombek DA. Ghrelin effects on the circadian system of mice. *J Neurosci.* 2007; 27:2890–5. [PubMed: 17360911]
64. Briatore L, Andraghetti G, Cordera R. Effect of two fasting periods of different duration on ghrelin response to a mixed meal. *Nutr Metab Cardiovasc Dis.* 2006; 16:471–6. [PubMed: 17015184]
65. Figlewicz DP, Lacour F, Sipols A, Porte D Jr, Woods SC. Gastroenteropancreatic peptides and the central nervous system. *Annu Rev Physiol.* 1987; 49:383–95. [PubMed: 3551807]
66. Torres-Farfan C, Richter HG, Rojas-Garcia P, et al. mt1 Melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin. *J Clin Endocrinol Metab.* 2003; 88:450–8. [PubMed: 12519889]
67. Valenzuela FJ, Torres-Farfan C, Richter HG, et al. Clock gene expression in adult primate suprachiasmatic nuclei and adrenal: is the adrenal a peripheral clock responsive to melatonin? *Endocrinology.* 2008; 149:1454–61. [PubMed: 18187542]
68. Wilkinson CW. Circadian clocks: showtime for the adrenal cortex. *Endocrinology.* 2008; 149:1451–3. [PubMed: 18359750]
69. Inokuchi A, Oomura Y, Nishimura H. Effect of intracerebroventricularly infused glucagon on feeding behavior. *Physiol Behav.* 1984; 33:397–400. [PubMed: 6514829]

70. Davidson AJ, Stephan FK. Plasma glucagon, glucose, insulin, and motilin in rats anticipating daily meals. *Physiol Behav.* 1999; 66:309–15. [PubMed: 10336159]
71. So AY, Bernal TU, Pillsbury ML, Yamamoto KR, Feldman BJ. Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. *Proc Natl Acad Sci USA.* 2009; 106:17582–7. [PubMed: 19805059]
72. Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. *Cell.* 2005; 122:803–15. [PubMed: 16143109]
73. Shimabukuro M, Koyama K, Chen G, et al. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci USA.* 1997; 94:4637–41. [PubMed: 9114043]
74. Schwartz MW, Baskin DG, Bukowski TR, et al. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. *Diabetes.* 1996; 45:531–5. [PubMed: 8603777]
75. Xu B, Kalra PS, Farmerie WG, Kalra SP. Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. *Endocrinology.* 1999; 140:2868–75. [PubMed: 10342879]
76. Revel FG, Masson-Pevet M, Pevet P, Mikkelsen JD, Simonneaux V. Melatonin controls seasonal breeding by a network of hypothalamic targets. *Neuroendocrinology.* 2009; 90:1–14. [PubMed: 19451698]
77. Peschke E, Peschke D. Evidence for a circadian rhythm of insulin release from perfused rat pancreatic islets. *Diabetologia.* 1998; 41:1085–92. [PubMed: 9754828]
78. Akiyama M, Yuasa T, Hayasaka N, Horikawa K, Sakurai T, Shibata S. Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. *Eur J Neurosci.* 2004; 20:3054–62. [PubMed: 15579160]
79. Castaneda TR, Tong J, Datta R, Culler M, Tschop MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol.* 2010; 31:44–60. [PubMed: 19896496]
80. Blum ID, Patterson Z, Khazall R, et al. Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience.* 2009; 164:351–9. [PubMed: 19666088]
81. Mistlberger RE, Marchant EG. Enhanced food-anticipatory circadian rhythms in the genetically obese Zucker rat. *Physiol Behav.* 1999; 66:329–35. [PubMed: 10336162]
82. Kudo T, Akiyama M, Kuriyama K, Sudo M, Moriya T, Shibata S. Night-time restricted feeding normalises clock genes and *Pai-1* gene expression in the db/db mouse liver. *Diabetologia.* 2004; 47:1425–36. [PubMed: 15309293]
83. Murakami DM, Horwitz BA, Fuller CA. Circadian rhythms of temperature and activity in obese and lean Zucker rats. *Am J Physiol.* 1995; 269 (Pt 2):R1038–43. [PubMed: 7503289]
84. Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, Yanagisawa M. Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. *J Neurosci.* 2004; 24:10493–501. [PubMed: 15548664]
85. Abe H, Kida M, Tsuji K, Mano T. Feeding cycles entrain circadian rhythms of locomotor activity in CS mice but not in C57BL/6J mice. *Physiol Behav.* 1989; 45:397–401. [PubMed: 2756028]
86. Abe H, Honma S, Honma K. Daily restricted feeding resets the circadian clock in the suprachiasmatic nucleus of CS mice. *Am J Physiol Regul Integr Comp Physiol.* 2007; 292:R607–15. [PubMed: 16990494]
87. Sheward WJ, Maywood ES, French KL, et al. Entrainment to feeding but not to light: circadian phenotype of VPAC2 receptor-null mice. *J Neurosci.* 2007; 27:4351–8. [PubMed: 17442819]
88. Feillet CA, Ripperger JA, Magnone MC, Dulloo A, Albrecht U, Challet E. Lack of food anticipation in *Per2* mutant mice. *Curr Biol.* 2006; 16:2016–22. [PubMed: 17055980]
89. Challet E, Mendoza J, Dardente H, Pevet P. Neurogenetics of food anticipation. *Eur J Neurosci.* 2009; 30:1676–87. [PubMed: 19863658]
90. Pendergast JS, Nakamura W, Friday RC, Hatanaka F, Takumi T, Yamazaki S. Robust food anticipatory activity in *BMAL1*-deficient mice. *PLoS One.* 2009; 4:e4860. [PubMed: 19300505]
91. Pitts S, Perone E, Silver R. Food-entrained circadian rhythms are sustained in arrhythmic *Clk/Clk* mutant mice. *Am J Physiol Regul Integr Comp Physiol.* 2003; 285:R57–67. [PubMed: 12649127]

92. Storch KF, Weitz CJ. Daily rhythms of food-anticipatory behavioral activity do not require the known circadian clock. *Proc Natl Acad Sci USA*. 2009; 106:6808–13. [PubMed: 19366674]
93. Lamia KA, Sachdeva UM, DiTacchio L, et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science*. 2009; 326:437–40. [PubMed: 19833968]
94. Pizzo MJ, Crystal JD. Representation of time in time-place learning. *Anim Learn Behav*. 2002; 30:387–93. [PubMed: 12593330]
95. Gibbon, J.; Fairhurst, S.; Goldberg, B. Cooperation, conflict and compromise between circadian and interval clocks in pigeons. In: Szabadi, CBaE, editor. *Time and Behaviour: Psychological and Neurobehavioural Analyses*. Elsevier Science; Amsterdam: 1997. p. 329-384.
96. Balsam P, Sanchez-Castillo H, Taylor K, Van Volkinburg H, Ward RD. Timing and anticipation: conceptual and methodological approaches. *Eur J Neurosci*. 2009; 30:1749–55. [PubMed: 19863656]
97. Stephan FK, Becker G. Entrainment of anticipatory activity to various durations of food access. *Physiol Behav*. 1989; 46:731–41. [PubMed: 2602500]
98. Yoshihara T, Honma S, Mitome M, Honma K. Independence of feeding-associated circadian rhythm from light conditions and meal intervals in SCN lesioned rats. *Neurosci Lett*. 1997; 222:95–8. [PubMed: 9111737]
99. Sclafani A. Learned controls of ingestive behaviour. *Appetite*. 1997; 29:153–8. [PubMed: 9344424]
100. Woods SC. The control of food intake: behavioral versus molecular perspectives. *Cell Metab*. 2009; 9:489–98. [PubMed: 19490904]
101. Froy O. Metabolism and circadian rhythms – implications for obesity. *Endocr Rev*. 2010; 31:1–24. [PubMed: 19854863]

Figure 1.

Left panel: circadian changes in plasma corticosterone levels in free-feeding (FF) and restricted-feeding (RF) rats, expressed as means $\pm$ SEM. * $P < 0.05$, significantly different, compared with all other time points in FF rats. # $P < 0.05$, significantly different, compared with all other time points in RF rats. Adapted from reference²⁸. Right panel: effects of meal duration on plasma corticosterone level in rats under restricted feeding. Plasma hormone levels are shown for rats fed ad libitum or for 2 weeks for 0.5, 2, and 6 h of restricted feeding per day. Meal time is indicated by rectangle. Values are means $\pm$ SEM. Adapted from reference³⁷.

Figure 2.

Upper panel: low- and high-magnification views of the stomach oxyntic cells, and localization of ghrelin and PER proteins. (a, upper) Image of the mouse stomach, indicating the corpus region where tissue was harvested (scale bar: 500 microns). (lower) Photomicrograph of a cross-section of the stomach wall (scale bar: 100 microns) (b) Photomicrographs of the stomach oxyntic gland stained for ghrelin, PER1 (upper), or PER2 (lower) and the overlay in optical sections (z axis = 2 microns) (scale bar: 10 microns). Adapted from reference²⁶. Lower panel: Comparison of ghrelin immunoreactive cells in wild-type and *mPer1, mPer2* clock mutant animals. Number of oxyntic cells expressing ghrelin in animals housed in light:dark (LD) and then placed in constant dark (DD) for 2 days and subsequently fed ad libitum (left), food-deprived for 24 h (middle), or food-restricted (right). * $P < 0.05$. Adapted from reference²⁶.

Figure 3.

Running wheel behavior of wild-type and ghrelin receptor-knockout mice during ad libitum feeding, food restriction, and food-deprivation conditions. (a) The bar above the actograms shows the light–dark cycle; time of food availability is shown in gray. Actograms depict activity of representative ghrelin receptor positive ($GHSR^{+/+}$) and ghrelin receptor knockout mice ($GHSR^{-/-}$) during ad libitum feeding (days 1–4), food restriction ZT6–14 (days 4–15), ad libitum food availability (days 15–18), and food deprivation (day 19). (b) Group activity profiles show the amount of wheel running during the last 7 days of restricted feeding in $GHSR^{+/+}$ (black) and $GHSR^{-/-}$ (gray) mice. The data are plotted in 10-min bins (mean $\pm$ SEM). $**P < 0.002$, difference between $GHSR^{+/+}$ and $GHSR^{-/-}$ in onset time of activity. (c) Line graph of cumulative wheel-running activity (mean $\pm$ SEM) from lights on (ZT0) to time of food presentation (ZT6) shows that $GHSR^{-/-}$ mice (solid gray line) ran 42.4% less than $GHSR^{+/+}$ (solid black line) mice. Superimposed are the curves derived from the Gaussian function (dashed lines). Cumulative wheel running data for individual $GHSR^{+/+}$ (d) and $GHSR^{-/-}$ (e) animals. The SEs for group data in C around each point reflect the differences among individual animals (d,e) in thresholds for initiating activity. The data, averaged over the 7 last days of food restriction, show that once that “go” decision is made, the animal continues to run as feeding time approaches, suggesting a change in threshold for the response. Adapted from reference²⁶.

Figure 4.

Effect of restricted feeding (RF) on distribution of food-related anticipatory (diurnal) and SCN-related (nocturnal) locomotor activity. Left, representative actogram of the locomotor activity of a wild-type mouse subject to a restricted feeding period (grey bar). Right, averaged activity count per hour of a free-feeding period (FF) and on day 10 of the restricted-feeding interval (five to six mice per time point). Adapted from reference⁷⁸. * $P < 0.05$.

Figure 5.

Left: Group average waveforms of activity at the food bin during the last 6 days of restricted feeding (RF) in the obese (thick line) and lean (thin line) Zucker rats. The data were collected in 10-min bins and were smoothed using a running average. The shaded bar indicates the feeding time. From reference⁸¹. Right: Decreased RF-induced food-anticipatory activities (FAA) in orexin neuron-ablated orexin/ataxin-3 mice. Note that the onset of the behavior was delayed and reduced in amplitude in the mutant animals compared to controls. From reference⁷⁸.

Figure 6.

Cartoon depicting the possible ways in which output signals from food-entrainable oscillators and mutations in circadian clock genes can influence the timing of food-anticipatory behaviors. Depicted are shifts in phase, amplitude, period, and threshold. The solid bracket depicts the initial state of the oscillator, and the dashed bracket shows the effect of the hypothetical output signal. Examples of each of these effects are shown in empirical data in Figures 2–5.

Figure 7.

Left: Comparison of mean and variability ($\pm$ SEM) of food-anticipatory activities (FAA) displayed as % daily total activity and as total daily wheel running counts, in animals housed in light:dark (LD) (a) and constant dark (DD) (b). Note that variability is much greater in DD than in LD. Activity corresponding to the 3-h FAA period is averaged over the last 5 days of ad libitum (AL) feeding and over days of food restriction (FR), shown on the x-axis. Left: *Differences between AL and other stages for wild-type and clock-mutant animals; †differences between genotypes within a stage. *, † $P < 0.05$; **, †† $P < 0.001$. Right: †The LD FR (1–5) stage is significantly different from LD FR (11–15), and the DD AL stage is different from DD FR (1–5) when the data are collapsed across genotypes. † $P < 0.05$; †† $P < 0.001$. No other genotypic or stage differences are significant. Adapted from reference⁹¹.

Figure 8.

Double-plotted record of wheel-running activity in suprachiasmatic nucleus (SCN)-lesioned rat subject to restricted feeding (RF) under light:dark (LD, left) and under dim constant light (LL, right). The vertical lines indicate the start and end of the food-availability interval (10.00–14.00 hours). Upon the termination of restricted feeding, animals were repeatedly allowed ad libitum feeding for 7–10 days and then exposed to food deprivation (FA) for 3 days. The LD cycle is indicated by the black bars on the bottom of the left panel. Adapted from reference⁹⁸.