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## The temporal organization of ingestive behaviour and its interaction with regulation of energy balance

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Review

# The temporal organization of ingestive behaviour and its interaction with regulation of energy balance

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## Abstract

Body weight of man and animals is under homeostatic control mediated by the adjustment of food intake. It is discussed in this review that besides signals reporting energy deficits, optimized programs of body clocks take part in feeding behaviour as well. Circadian light- and food-entrainable clocks determine anticipatory adaptive behavioural and physiological mechanisms, promoting or inhibiting food intake. In fact these clocks form the constraints within which the homeostatic regulation of feeding behaviour is operating. Therefore, a strong interaction between circadian and homeostatic regulation must occur. In this homeostatic control, a wide variety of regulatory negative feedback mechanisms, or satiety signals, play a dominant role. In this respect several gut hormones and body temperature function as 'short-term' satiety factors and determine meal sizes and intermeal intervals. Leptin, secreted by fat cells in proportion to the size of adipose tissue mass, is probably an important determinant of the 'long-term' regulation of feeding behaviour by setting the motivational background level for feeding behaviour. Thus, initiation or termination of meals at any particular point in time, depends on the resultant of all satiety signals and on constraints imposed by circadian light- and food-entrainable oscillators. © 2002 Elsevier Science Ltd. All rights reserved.

*Keywords:* Circadian rhythms; Regulation of food intake; Body weight; Leptin; GLP-1; Cholecystokinin; Hypothalamus; Food-entrainable oscillator; Suprachiasmatic nucleus

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## 1. Introduction

Food intake in most species including humans and rodents is subject to homeostatic control such that the

total amount of absorbed energy equals energy expenditure throughout a large part of the adult life. This homeostatic control is an example of a very precise regulatory system because the slightest, yet, persistent mismatch between daily energy intake and expenditure could lead to dramatic alterations in body weight in the long run [1]. The fact that energy intake is regulated to

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serve energy balance is reported in a seminal paper by Adolph [2] showing that rats subjected to dilution of their diet during a period of several weeks with a non-caloric substance responded with an appropriate increase in food intake essentially rendering their energy balance undisturbed. The opposite response was observed when rats were subjected to a diet with a higher caloric density. By that time, Hetherington and Ranson had already pioneered the concept that the hypothalamus plays a crucial role in the maintenance of energy balance [3]. They found that electrolytic lesioning of the ventromedial portion of the hypothalamus (VMH) causes rats to become dramatically hyperphagic and obese whereas a lesion in the lateral hypothalamus (LH) produces the opposite. This idea was later confirmed and extended by Anand and Brobeck [4]. These and other observations have led several investigators [5–7] to propose a number of factors reporting energy content in the body to the CNS.

There is no doubt that the combined use of genetically modified animals and the development of modern molecular biology over the last decade have dramatically expanded our knowledge about the mechanisms that regulate energy balance. However, relatively little attention has been given to the behavioural constraints within which regulation of energy balance is operating. The most obvious one that has a major influence on energy balance is, of course, the temporal organization of ingestive behaviour throughout the daily cycle. Many higher animal species, including humans and rodents eat their food in specific bouts (i.e. meals) that are interspaced by time intervals of different durations, with the longest interval usually coinciding with the inactive period (i.e. when most animals sleep). From a teleological standpoint, these intermeal-intervals could have the advantage to allow some animals to cope better with the environment compared to the condition when they would be feeding continuously. In nature this might have been crucial for survival of some species, and thus could ultimately have influenced the evolutionary success of that species. Many animals, with the rat as prototypical example, continue to display temporal feeding patterns under standard laboratory conditions, even when they have been inbred for many generations. There is growing evidence that specialized ‘body clocks’ are involved in the temporal organization of ingestive behaviour. Since rats housed under laboratory conditions also defend their energy balance (i.e. with relative vigour depending on the strain used), one implication is that there should be a strong interaction between these ingestive clock programs and the mechanisms that regulate energy balance. It is this interaction which is the main focus of the present review with the rat as animal model used to discuss general principles.

## 2. Circadian timing system of ingestive behaviour

### 2.1. Ingestive behaviour at dusk

As mentioned above, most animals have an active and

inactive phase coupled to circadian rhythmicity. In the case of the rat and many other rodent species, most of the behavioural activities are organized during the dark phase, whereas sleep occurs usually during the light phase. This is equally the case when they are housed under the above mentioned ‘standard laboratory conditions’ (i.e. 12 h light/12 h dark-cycles, individual housing, ad libitum access to food and water provided by hopper and bottle, respectively). While they generally schedule most of their food intake in the darkness with peaks at the beginning (dusk) and towards the end (dawn) of the night, each rat has its own unique meal pattern, which is more or less repeated from day to day [8]. In one experimental paradigm, we have attached a relatively dark burrow (nest box) to their cages, with the entrance on the opposite side of where the food hopper and water bottle are located. During the light phase the rats prefer to stay inside this nest box. At the onset of the dark phase, however, the rats come immediately out of the nest box and display preference for the larger arena where they typically eat and drink in front of the food hopper and water bottle, respectively. Rats occasionally make an excursion from the nest box to the food hopper/water bottle during the light phase, but they quickly return with every bite to the nest box where they either ingest the obtained food or hoard it for later consumption. The duration of these visits to obtain food and water during the light phase is inversely related to the brightness of the light, suggesting that nocturnal animals are avoiding light to a certain extent [8]. One implication of these observations could be that ‘light-avoiding behaviour’ might play a role in the expression of the daily rhythmicity of ingestive behaviour. To investigate this hypothesis, rats were subjected to a so-called ‘skeleton photoperiod’ (SPP), in which total darkness is interrupted by light pulses (15–30 min) at the original changes from light to dark and vice-versa. This method leaves the endogenous component of light-entrained behaviour intact. With the switch from the daily light–dark (LD) cycle to SPP (Fig. 1), the rats started to eat on average only one meal more during the subjective day (the previous light phase under LD) of the SPP condition, which indicates that light avoidance only plays a minor role in the expression of the daily rhythmicity in ingestive behaviour [8].

In the normal LD situation, the onset of the dark phase itself is a particularly potent stimulus for ingestive behaviour, since most rats generally eat one large meal soon after lights are off, even if the LD cycle is suddenly 6 h advanced. A further concentration of feeding activity, which usually commences about 2 h after the first meal in the dark phase, takes approximately 1 week to adjust to the new 6-h advanced LD cycle (Fig. 1) [9]. The immediate darkness-induced feeding is probably caused by an energy deficit incurred during the light phase in which rats hardly eat. The subsequent peak in feeding activity at 2 h in the dark phase is likely the result of an ‘endogenous oscillator’ or ‘body clock’, which is only slowly adjusting to sudden

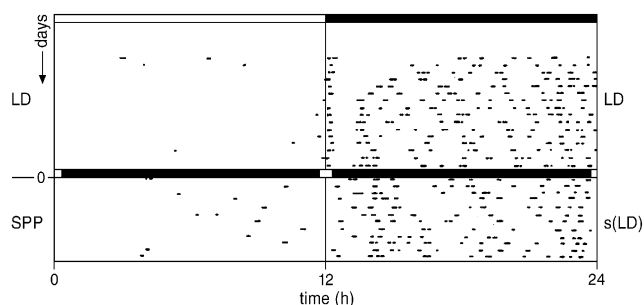


Fig. 1. Daily meal pattern of one representative rat. The lighting conditions were changed from LD to SPP on day 0. The black horizontal bars, indicate the time that lights are switched off (from Ref. [9]).

phase-shifts in the LD cycle. These slow adjustments are also observed in rats subjected to phase shifts in the SPP condition, suggesting that light pulses can act as ‘Zeitgebers’; i.e. cues able to entrain and synchronize endogenous oscillators governing the daily rhythm of ingestive behaviour. When these light pulses are absent, for instance in continuous light (LL) or continuous dark (DD), rats are a priori unable to synchronize their oscillators to the environment, and the behavioural expression is controlled by the ‘endogenous oscillators’ alone. A so-called circadian-free running rhythm develops with a daily period which is close to that of one single earth rotation (*circa* = approximately, *dies* = day). These periods may be shorter or longer than 24 h. The length of the period depends on the species and on the individuals. There is some evidence that day-active animals on average have shorter periods than night-active animals [10].

## 2.2. Ingestive behaviour at dawn

Whereas the first meals at dusk are probably aimed at replenishing depleted energy stores, the dawn peak of food intake might enable the rat to ensure energy availability during the ensuing light phase when ingestive behaviour is suppressed. We have hypothesized that, analogous to the situation at dusk, the dawn peak in ingestive behaviour is also governed by endogenous circadian pacemakers. The following experiments were performed to shed light on this point. A group of rats was fed ad libitum and housed under a LD (12:12) rhythm. From a certain day on, they had their food continuously removed during the last 2 h of the dark phase, which prevented the occurrence of the typical peak in ingestive behaviour at dawn. The rats never advanced their ingestive dawn peak to the final hours before food removal, but instead they compensated by eating more in the light phase. In another experiment where food availability remained ad libitum, but from a certain day on the light was continuously switched on during the final 2 h of the dark phase (thus yielding a LD cycle of 14:10), the rats now advanced their dawn peak by 2 h already after a single experience of the new regime. The conclusion is that the dawn peak is not governed by fluctuations in energy content

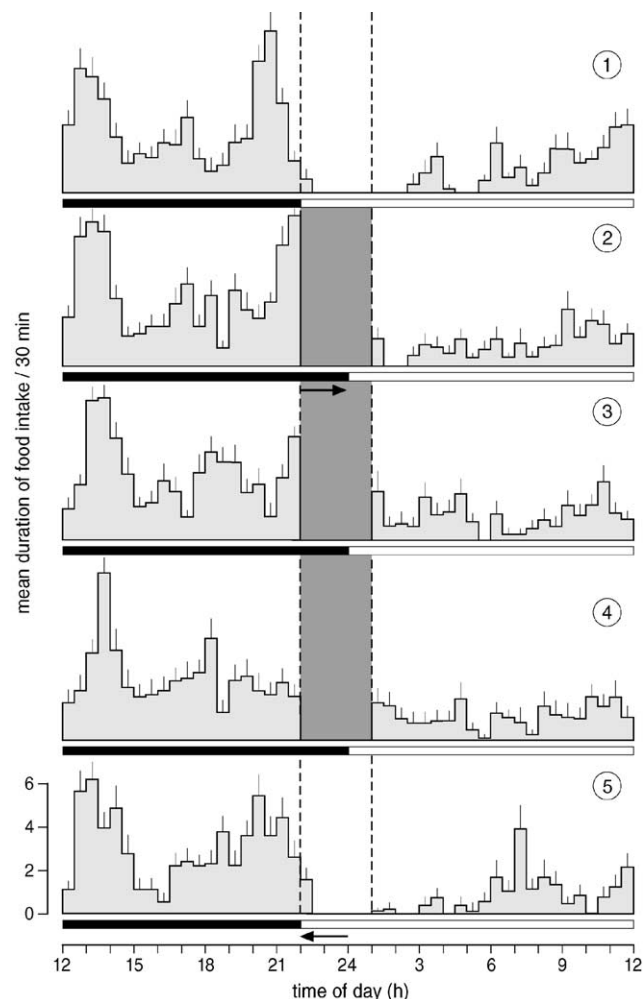


Fig. 2. Mean duration of food intake per 30 min for week 1 (control on LD 14:10), 2, 3 and 4 on delayed light onset with food restriction at dawn, and week 5 (postexperimental week with advanced light onset, from LD 12:12 to LD 14:10). Week numbers are indicated on the right side of the figure panels. Periodic food restriction at dawn is indicated by the rectangular dark grey area. The shift in light onset is indicated by the arrow and the horizontal black bar indicates the dark phase (from Ref. [12]).

of the body, but by the experience of the LD change itself [11]. It is therefore tenable to suggest that an endogenous program might mediate anticipation of feeding before the onset of light. This is even clearer from an experiment that started on a schedule of LD 14:10 (Fig. 2, week 1).

A sudden extension of the dark phase yielding a continuous regime of LD 12:12 with simultaneous food deprivation over the last 2 h of the dark phase resulted in a gradual disappearance of the dawn peak (Fig. 2, weeks 2, 3 and 4) [12]. However, the motivation to feed at dawn persisted under these conditions, since rats immediately showed the dawn feeding peak upon return of food (not shown in this figure) [12]. In this experiment, the rats apparently compensated for their caloric deficit by eating early in the light phase, when in nature the predation pressure would be high. The rapid shift in dawn feeding upon changes in light onset and food restriction, and its

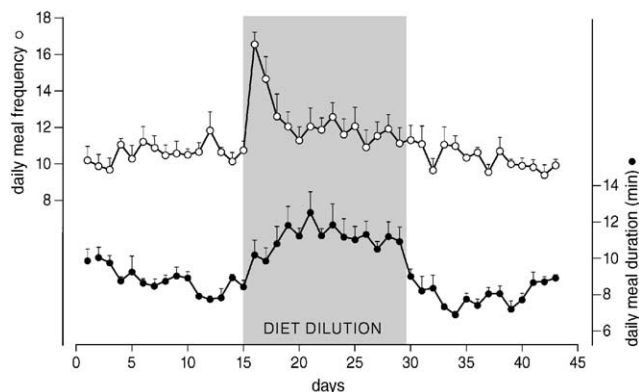


Fig. 3. Mean daily number and size of meals before, during and after caloric dilution of the diet (from Strubbe and Van Dijk, unpublished observations).

quick reappearance after discontinuing food restriction, argue against habit formation by learning, as being responsible for the occurrence and maintenance of dawn feeding. Therefore, the onset of daytime is probably preprogrammed in an endogenous clock to feed at least 1–2 h in advance and return in the burrow before light intensity increases. It means therefore that animals possess an ‘anticipation capacity’ of how long it allows them to eat before light onset. The question remains, however, is the circadian system responsible for the initiation of dawn feeding? The following may give some additional support for this hypothesis. Observations in humans and in rats show that circadian pacemaker activity is gradually reduced during aging, causing a decrease in amplitude of several circadian rhythmicities [13]. When aged rats were submitted to SPP where masking properties of light were removed, this yielded an arrhythmic feeding pattern with a particularly pronounced decline of dawn feeding, and a subjective light phase with higher feeding activity [13] relative to young rats subjected to SPP.

### 2.3. Maintenance of energy balance: meal frequency versus meal size

It has been discussed in Section 1 that animals can adapt easily to changes in energy content of the diet [2] so that the daily energy intake equals energy expenditure, and thus essentially leaving body weight unchanged. In the case of diet dilution, rats have the option to increase their intake by either initiating more meals, by increasing meal size, or both. In one of our experiments, rats fed ad libitum on normal lab chow ate on average 10 meals over the daily cycle (Fig. 3). When they were suddenly switched to a diet diluted by cellulose (at the onset of the dark phase) now yielding 75% of the energy/gram relative to the normal lab chow, these rats increased the number of meals to an average of 15 during the first day of diluted diet exposure. The increase in meal number was particularly pronounced at about 6 h after feeding on the new diet. Over the following 3 days, however, there was a gradual decline in the number of

meals to finally the same number as controls on the fourth day after the diet switch. Over the same period, however, the opposite occurred with meal duration (which correlates almost completely with meal size under a variety of conditions [13]); i.e. the average meal duration started to increase to finally about 25–30% longer than rats on the control chow (Strubbe and Van Dijk, unpublished observation). When switching these rats from the diluted diet (at day 14) back to the normal chow, meal duration immediately returned to the same length as that of controls.

Thus, these data suggest that reduction in energy density of the diet is adequately compensated mainly by increasing meal size, thereby maintaining energy balance and rendering body weight unaltered. The point here is that rats (and this may be relevant for many other species) apparently prefer a certain amount of meals over the circadian cycle irrespective of the energy content of the diet. In fact, the temporal organization of meals before diet dilution and the one that rats displayed after a few days on the diluted diet are very similar (Fig. 3). Therefore, the entrainable oscillator involved in the temporal organization of meals is quite powerful, and apparently forms the constraints within which the long-term control of energy balance is acting. The observation that the switch from compensation by meal frequency to meal duration took about 3–4 days is quite intriguing, particularly in light of the fact that rats are able to sense the reduced energy density of the diet already within 6 h. To equalize the temporal patterning of intake of the diluted diet to that of the undiluted diet, perhaps animals have to ‘learn’ to eat larger meals before it allows them to reduce meal frequency. Thus, the increase in meal frequency of a diluted diet might be regarded as a ‘compensatory’ response induced by a mismatch between the ‘anticipated’ and the actually ingested amount of energy. When animals have succeeded to increase their meal size, the anticipated and ingested amount of energy are balanced again. The idea that anticipatory responses are generally preferred above compensatory responses might apply to many other physiological and behavioural mechanisms, and could obviously have major advantages in terms of controllability of whatever needs to be regulated.

## 3. The suprachiasmatic nucleus and regulation of food intake

### 3.1. The suprachiasmatic nucleus

The experiments described above indicate that circadian rhythms in behaviour including feeding are generated somewhere in the body with the central nervous system as a major candidate. The suprachiasmatic nucleus (SCN), a small area above the chiasma opticum was finally identified as a master clock generating many circadian rhythms in mammals [14]. Lesioning of this area causes immediate disruption of the circadian rhythm of food intake, as is

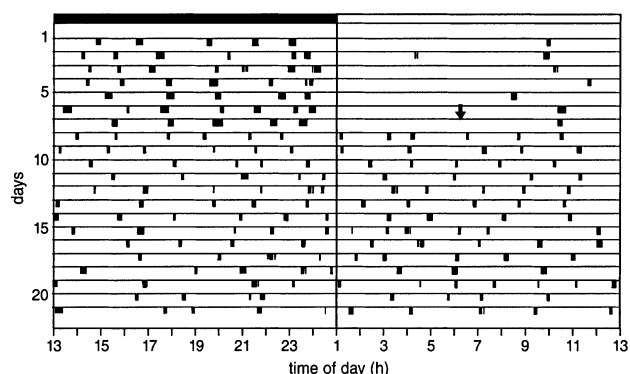


Fig. 4. Daily pattern of feeding during successive days in one representative rat. On day 7, the arrow marks the time of electrolytic lesion of the suprachiasmatic nucleus. The horizontal black bar indicates the dark phase (from Ref. [15]).

shown in Fig. 4 [15]. The lesion does not induce blindness, since the animals continue to respond to visual stimuli (among which, for example, the phenomenon of the ‘short-visits’ to the food hopper during the light phase is indicative).

A lesion in the SCN also disrupts the circadian rhythm of many other behaviours and physiological processes [15]. While the master circadian pacemakers appear to be located within the SCN, there is evidence that many other areas contain sub-oscillators that are also involved in generating rhythmic processes which are superimposed on the circadian rhythm. Among them is the ultradian rhythm, which has a periodicity of a few hours [16]. Also the pineal gland has been implicated in circadian organization of behaviour, but the exact mechanism is still under investigation. There is increasing evidence that pinealectomized rats entrain their rhythms faster than intact control rats, suggesting that the pineal gland, probably via its secreted hormone melatonin fulfils a stabilizing function on the circadian rhythm governed by the SCN [17]. Because the circadian pacemaker in the SCN influences many behavioural and physiological processes, the pathways from the SCN to other brain areas and reverse have been subject of many investigations during the last decades. The presence of at least 20 different neurotransmitters and neuropeptides in perikarya and axons within the SCN is evidence for a huge exchange of information between the SCN and other brain areas [18]. The synthesis and release of these neurotransmitters may be controlled by several recently discovered clock genes (for review see Ref. [19]). Electrophysiological recordings of unit activity in the SCN show the presence of neurons which are active either during the dark phase or during the light phase [20]. The circadian rhythmicity within the SCN remains present even when all other nervous connections are disrupted [21]. This self-generated rhythm is not unique for the central nervous system, since it can also be found in several peripheral endocrine/metabolic tissues, such as for example, insulin producing pancreatic B cells [22] and the liver [23]. The

SCN, however, seems to be re-entrainable by information from the environment to synchronize their activity. The experiment described in the previous paragraph indicates that this information is provided by light, which reaches the SCN neuronal cell bodies via the retina and the retino-hypothalamic tract [20].

Because the circadian pacemaker in the SCN transfers information to other brain regions which may possess sub-oscillators with endogenous programs for sleep, feeding and drinking behaviour and autonomic functions, it is believed that SCN has a dramatic impact on the energy fluxes over the circadian cycle. SCN-lesioned rats are nevertheless capable of maintaining energy balance in the long run. The SCN has many projections to the dorsomedial hypothalamus (DMH) and the paraventricular nucleus (PVN) from which several autonomic and neuroendocrine functions are controlled [18,22]. The PVN is also a major centre involved in the circadian control of food choice [24]. The classical feeding areas, i.e. the VMH and LH hypothalamus are probably also links in the chain of projections from the SCN. Although relatively small lesions within the VMH produce dramatic alterations in body weight, the circadian rhythmicity of ingestive behaviour remains intact (Strubbe, unpublished observations). On the other hand, a large lesion in the VMH but without damaging any part of the SCN results in a loss of circadian rhythmicity in food intake [25]. It may be suggested that a large VMH lesion ablates pathways from the SCN to the DMH and that the VMH itself is probably not linked in the chain of neuronal connections from the SCN which affects circadian rhythmicity in food intake. An alternative possibility could be that these rats are so dramatically hyperphagic that they have shifted some of their food intake to the light phase. However, studies in genetically obese Zucker rats, which are hyperphagic, do not support the view that obesity is associated with loss or disturbance of circadian rhythmicity [16].

### 3.2. Memory for feeding time: the ‘food-entrainable oscillator’

Ingestive behaviour is usually associated with food-anticipatory behaviour and expresses itself, at least in the rat, as increased locomotor behaviour before food consumption [26]. Besides the increased locomotor behaviour, there is probably also a change in neuroendocrine/autonomic output, which affects metabolism independent of locomotor behaviour. These effects are particularly pronounced when rats are kept on a limited food access schedule of, for instance, 1 h/day. During this hour, rats readily learn to eat the amount of food necessary to cover their total daily energy requirements and are thus no less capable of maintaining normal energy balance relative to animals feeding ad libitum [27]. The increased locomotor activity advancing the time of return of food persists a few days even when no food is given, but gradually disappears when ad libitum conditions are reinstated, and the same is

Table 1

Pre- and postprandial correlations before and after lesioning of the suprachiasmatic nucleus during day and night. No data were obtained during daytime in the non-lesioned group since there were almost no meals and intervals. Kendall's rank correlation test. *n*, number of meals and intervals, and correlation coefficient. From Strubbe and Dijkstra (unpublished observations)

	Light phase (after lesion)		Dark phase			
	Pre	Post	Before lesion		After lesion	
			Pre	Post	Pre	Post
Rat #1						
<i>n</i>	14	16	26	29	21	21
Coefficient	0.34	0.60**	0.01	0.14	0.18	0.65**
Rat #2						
<i>n</i>	19	19	29	30	18	19
Coefficient	0.27	0.64**	0.12	0.24	0.14	0.54**
Rat #3						
<i>n</i>	12	14	29	26	20	19
Coefficient	0.30	0.47**	0.01	0.23	0.10	0.56**

\*\**p* < 0.01.

probably true for the associated neuroendocrine/autonomic and metabolic correlates. There is certainly a learning component to this phenomenon but the precise reference for locating the time to anticipate feeding is not known. When SCN lesioned rats (with otherwise obliterated circadian rhythmicity under ad libitum conditions) are subjected to limited food access of 1 h/day, they do not appear to have an impairment in displaying the food anticipatory activity (FAA) advancing the time of feeding relative to control animals [26–28]. This suggests that an extra-SCN entrainable oscillator(s) must exist which brings about this behaviour. Since FAA can be evoked independent of the timing of the LD cycle, and is not associated with any other behaviour except feeding, it has been suggested that these meal-associated rhythms are governed by a FEO [27]. Thus far, no central nervous structure has been identified to contain such an oscillator, but recent papers suggest that these may reside in peripheral tissues [22,29,30]. Even under continuous dark or light conditions rats show FAA without re-entraining other free-running rhythms. Yet, changing the length of LD cycle affects the FAA which suggests a weak coupling between the light-entrainable oscillator (LEO) and FEO. The precise function of FAA is unknown, but it may be suggested that it is aimed at increasing body temperature, which could increase the efficacy of enzymatic processes involved in nutrient handling. The FEO will adjust the physiological and behavioural responses adequately without necessarily phase-shifting the light entrainable circadian system [9]. Although the appearance of FEO is particularly evident under conditions of limited food access schedules, the FEO is probably also at work under more natural conditions of feeding activity. By optimizing digestive and metabolic

processes animals are able to concentrate the bulk intake of food and water into a small window of time to reduce the risk of being victim of predation.

### 3.3. Temporal organization of ingestive behaviour in SCN-lesioned rats

It was discussed above that lesioning of the SCN causes disruption of the circadian rhythm of food intake. SCN-lesioned rats nevertheless eat their meals in discrete bouts interspaced by time intervals of different duration. Besides the clear behavioural arrhythmia, SCN-lesioned rats display a higher meal frequency, particularly when the SCN lesion is relatively large. One possibility underlying this phenomenon might be that SCN-lesioned rats lack 'anticipation' capacity, which could lead to a situation where rats regulate food intake more adaptively. In that case, it might be expected that the timing of meals relies more on the current energetic status of the animal, instead of on the anticipated energetic status. Thus, since the energetic status largely depends on previous energy intake, one might hypothesize that the initiation/termination of meals in SCN-lesioned rats is determined by previously ingested food. To investigate this hypothesis, we have analysed interactions between meal size and intermeal intervals in SCN-lesioned rats, and found that meal size was tightly correlated to the postprandial, but not to the preprandial intermeal interval. These data therefore suggest that animals lacking an endogenous entrainable oscillator regulate the onset of each next meal as a function of the size of the previous meal (Table 1).

In the intact animals, a correlation between meal size and intermeal intervals was not observed suggesting that the coupling of previous energy intake to initiation of each next meal is weak and/or masked by the SCN effects on temporal organization of food intake patterns. It does not mean, however, that signals associated with ingested food are not important in control animals. For instance, infusions of liquid food in the gastrointestinal tract caused an increase in the intermeal interval. Moreover, these signals are probably very important to control the termination of every meal during which they are building up. This has been demonstrated, for example, in the situation of sham-feeding. Thus, rats with a fistula in the oesophagus or stomach through which ingested nutrients leave the body have a dramatically increased meal size relative to the situation in which the food remains inside the body [31]. Many factors associated with the energetic status could influence food intake, and the most important ones are discussed below.

## 4. Influence of signals related to energetic status on the temporal organization of ingestive behaviour

### 4.1. Signals associated with metabolic status of the body

It was discussed that rats subjected to diet dilution are

able to adapt to this new situation by increasing meal size. The fact that animals are able to sense the energy content of the diet reasonably fast and reschedule their ingestive behaviour, indicates that the amount of ingested energy is assessed. Although some data exist that the energy content of a diet can be assessed by orosensory or gastrointestinal processes [32], a major hypothesis states that the intracellular energy content of some specialized organs (e.g. liver, brown adipose tissue) or neuronal circuits (arcuate hypothalamic nucleus, VMH) can be signalled and relayed into CNS areas involved in regulation of food intake and energy balance. In that case, it would be predicted that a reduction in energy content in the body by increasing energy expenditure (provided that the dietary energy content is held constant) would have a similar predictable effect on food intake and its temporal organization as with reduced dietary energy content. As discussed below, we have studied a few examples of increased energy expenditure.

*Cold acclimatization.* When animals are placed in a cold (12 °C) environment, many warm-blooded species start shivering in an attempt to maintain euthermy. After a period of acclimatization to the reduced ambient temperature, they are able to maintain body temperature through non-shivering thermogenesis. This process takes place mainly in brown adipose tissue, and requires uncoupling of the proton-gradient across mitochondrial membrane during which heat is radiated [33]. The amount of energy lost in this process is appropriately compensated by an increase in food intake, mainly through increasing meal size but not through meal frequency (Strubbe and Van Dijk, unpublished observations). The opposite effect on meal size is observed when animals have adapted to an increased ambient temperature of 30 °C. Thus, taken together, these data suggest that changes in energy requirements to maintain euthermy are compensated by adequate changes in food intake, whereby body weight and energy balance are maintained [34–37].

*Diabetes mellitus.* Rats treated with streptozotocin lose pancreatic B-cell function rendering them void of insulin and type-I diabetic, and, if not treated, they are dramatically hyperglycaemic. The blood glucose level is usually higher than the kidney threshold for glucose, and the diabetic rat loses large amounts of energy in the form of glucose via the urine. These rats clearly compensate by increasing their food intake. The hyperglycemia obviously has major effects on several aspects of glucose and fat metabolism. Furthermore, they become lipodystrophic since insulin is a key hormone to store nutrients in the form of fat. The concomitant polyuria caused by osmotic diuresis forces the rats to drink a volume of 20 times more than non-diabetic rats. In spite of these dramatic disturbances, diabetic rats display a clear dusk and dawn peak of feeding activity and retain their other typical circadian characteristics of meal patterning [25]. As in the case of a drop in ambient temperature, the increased energy requirements are compensated for by increasing meal size and a small increment

of feeding during the light phase, whereas all other factors determining the meal pattern are unaffected.

*Lactation.* While the above mentioned studies show that with moderate changes in energy requirements total food intake is regulated through the regulation of meal termination, it is of interest to see how feeding behaviour changes with higher energy requirements. This was investigated by assessing the temporal organization of food intake of lactating rats feeding a litter of 10 pups [38]. Over the oestrous cycle food intake varied between a mean of 17 g in di-oestrus and 13 g in oestrus. There is some evidence that increased levels of estrogens are responsible for the suppression of feeding activity during oestrus. On the second day after conception, food intake was established at di-oestrus levels and remained high for 2 weeks. Body weight increased gradually during this period. From birth onward, food intake increased rapidly reaching an average of 58 g during the third week postpartum. During the first postpartum week, food intake rose from 20 to 40 g. This was accomplished through an increase in meal size, but not in meal frequency. During the second and third postpartum weeks, meal size did not rise further but now there was an increment in meal frequency. In particular, the increase in number of day-time meals was prominent. The typical bimodal pattern persisted over the three postconception and postpartum weeks [38]. These data suggest that, despite changes in the feeding strategy during the lactation period, thereby interfering with sleeping behaviour, no severe alteration of the circadian characteristics of the meal pattern occurred.

Taken together, the data mentioned above and in Section 2.3 suggest that animals are able to signal their energy status, and are able to compensate changes appropriately through changes in food intake. While this seems an example of a simple regulatory feedback loop, the mechanisms that are involved in this are still a matter of debate. A general idea put forward by Nicolaidis [39] and later by Friedman [40] and Ritter [41] proposes that energy availability (perhaps in the form of ATP or other basal energy carriers) is continuously monitored by the CNS. If energy availability declines, this would cause an increased motivation to eat. Indeed, a number of 'antimetabolite' drugs have been discovered (e.g. 2-deoxy-D-glucose, methyl-palmoxyrate, 2,5-anhydro-D-mannitol, etc.) which inhibit fuel metabolism and essentially reducing the formation of ATP, and these have profound orexigenic effects. While this sort of mechanism could play a role in the compensatory response to changes in energy availability or expenditure, they do not necessarily provide explanations for regulation of meal size. Another theory that takes meal size into account is the thermoregulatory control of food intake [7,35,36]. This theory proposes that meals are terminated when the body temperature reaches a certain nadir (Fig. 5).

One function of the prandial rise in temperature may be to optimize enzymatic processes in preparation for ingesting,



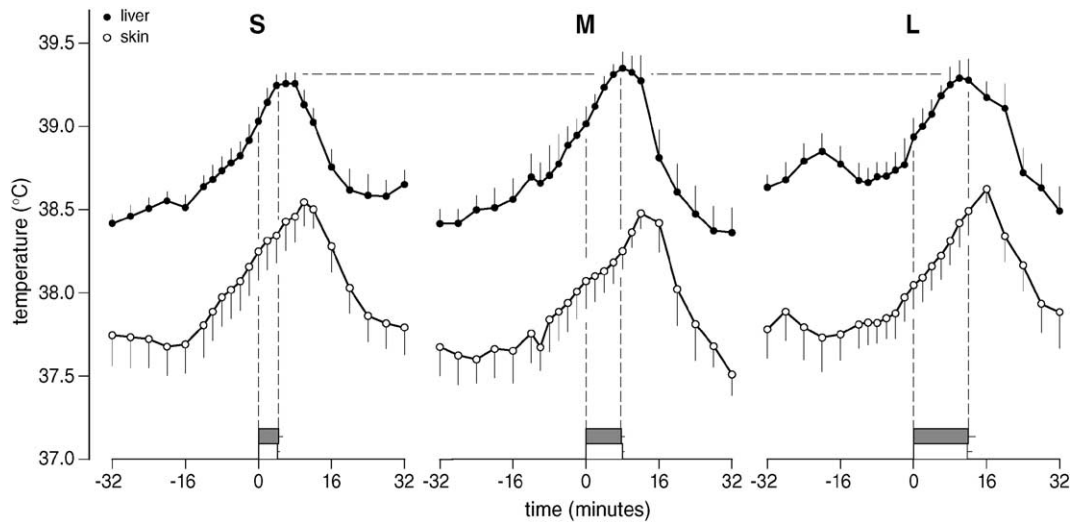


Fig. 5. Mean (+1 SE) hepatic and skin temperatures for separate groups of rats consuming relatively small (S), medium (M) or large (L) meals during the dark phase of the day/night cycle. The dashed horizontal bars (+1 SE) indicate the mean durations of the meals associated with the depicted hepatic temperatures (from Ref. [35]).

absorption, compartmentalization, and/or metabolism of ingested nutrients [35,36]. Another function may be to probe the energy status of the body [42]. Careful analysis of the increases in core temperature and meal sizes revealed that large meals coincide with a longer trajectory over which body temperature increases relative to small meals [35], suggesting that long temperature trajectories allow large meals. There might be a consequence with respect to living under extreme ambient temperatures. For example, one may speculate that the trajectory over which a rat's core temperature rises is longer in a cold environment, even when the thermogenic capacity is increased in rats adapted to cold. The outcome would be a larger meal. When rats live under relatively high ambient temperatures, the opposite would be expected to occur. The mechanism through which diabetic rats increase meal size probably relies on the lack of insulin. Thus, although hyperglycaemic, they lack insulin to transport the abundantly circulating glucose inside cells, which in turn might impair thermogenic capacity [43], and therefore prolong the distance of the temperature increment during intake of a meal. The possibility that glucose uptake might be important for thermogenesis in healthy animals is consistent with the fact that the level of blood glucose usually falls before a meal [44]. A somewhat similar case could be made for the lactating rat, but in this case it is not the lack of insulin, but the relative insulin insensitivity that underlies thermogenic incapacity. Since obese animals are frequently insensitive to insulin and have reduced thermogenic capacity as well, this could account for the increase in meal size as well.

#### 4.2. Signals associated with gastrointestinal loading

Before being made available for metabolic purposes, food has to pass the digestive tract where it is broken down

into smaller absorbable components. Over the last several years, a wide variety of signals from several compartments of the gastrointestinal system has been discovered that can affect ingestive behaviour and these are shortly reviewed below. Nutrient loading of the oropharyngeal cavity and to a lesser extent of the upper gastrointestinal tract (oesophagus, stomach) can evoke signals (taste, texture, distensions) that are directly aware to the consumer and generally relate to appreciation of the food eaten. Ingested nutrients can also stimulate the release of several peptide hormones, such as cholecystokinin (CCK), gastrin-related peptide, bombesin, glucagon-like peptide-1-amide (GLP-1) and others. Among these, CCK is regarded as the prototypical satiety agent [45, 46]. CCK released from the duodenum acts on the afferent pathways of the vagus nerve, and sub-diaphragmatic transection of the vagus nerve consistently abolishes the suppressive effects of CCK on food intake. Similar effects are observed when the nucleus tractus solitarius (NTS)—the terminal region for vagal nerve afferents—is damaged. Since CCK containing neurons and receptors are found in the entire pathway from intestine to the VMH, via the vagus nerve, the NTS, and the parabrachial nucleus, it is possible that CCK influences satiety at all these levels of organization [47]. Since lesioning of the VMH results in a hyperphagic rat, it has been suggested that this area is the main target for feedback control of satiety signals [3,37]. Gastrointestinal factors are secreted as a result of ingestion except ghrelin, a growth hormone secretagogue, secreted by the stomach [48]. Circulating levels of ghrelin peak just before each meal, and decline rapidly as the stomach fills. Ghrelin is believed to be involved in the control of ingestive behaviour since infusion of small amounts of ghrelin into the CNS consistently increase food intake [49].

The gastrointestinal feedback signals (with ghrelin secretion—as the exception—advancing nutrient loading)

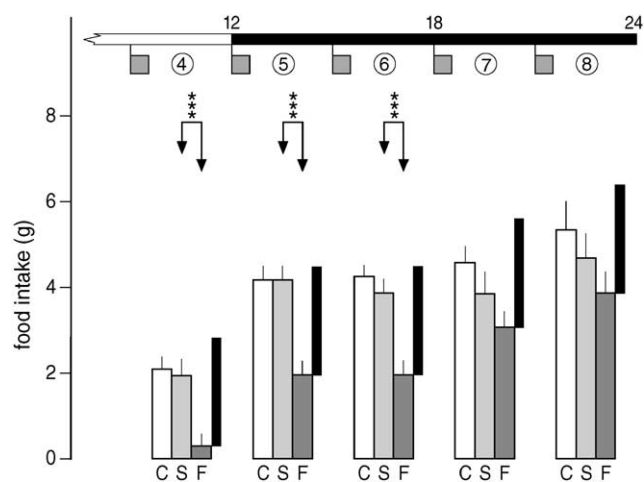


Fig. 6. Food intake (g) per 3 h period in controls, i.e. no infusion (C), solvent infusion (S) and food infusion (F). The black bar indicates the amount of liquid diet infused as grams of normal food yielding the same amount of energy. Black bar on top indicates the dark phase. The time of infusion is expressed as the grey bars on top.  $***p < 0.005$  (from Ref. [50]).

are greatly affected by circadian influences for the simple reason that ingestive behaviour is strongly affected by circadian influences. The question arises whether these gastrointestinal feedback signals (and the same is true for the earlier discussed thermoregulatory and metabolic signals) have fluctuating efficacies over the LD cycle. In other words, what is the contribution of the influence of the gastrointestinal signals or from metabolism in relation to the influence of the circadian pacemakers to the control of feeding? This was investigated by assessing food intake after infusion of liquid food directly into the stomach at different times of the LD cycle. This method of food administration avoids the possible influence of oral sensations. The study demonstrated that intragastric infusion of food is more effective in suppressing intake during the light phase and first part of the dark phase relative to the second half (Fig. 6). This effect is mainly due to a delay of the first meal after the infusion, by which the intermeal interval during the infusion period is increased. No significant effects on meal sizes were observed in various periods. These results suggest that mainly meal initiation is affected by the gradual infusion of food into the stomach and not the termination of meals [50]. The infusion, however, was started during the intermeal-interval. It can therefore not be excluded that it would affect meal size as well when the infusion was started during the meal. During the second part of the dark phase, the suppression of food intake is far less complete. In particular, during the last 3 h period the first meal after infusion was not even postponed. It might be concluded that signals reporting excess amounts of nutrients in the gastrointestinal tract at dawn are neglected or overruled by other signals forcing the rat to advance eating prior to the light phase, a period of aphagia and sleep. This possible anticipatory feeding during the end of the night is

supported by others who found that the stomachs of rats were strongly filled with food at the end of the night but not during the beginning of the dark phase [51].

Based on the results of experiments described, it was suggested that a circadian pacemaker, which is entrained by light onset, controls the occurrence and maintenance of the feeding peak at the end of the night. Thus the endogenous program in the circadian pacemaker increases feeding motivation at the end of the dark phase thereby overruling satiety signals reporting the energy content present in the gastrointestinal tract. This experiment shows clearly that in particular at the end of the dark phase the circadian pacemaker is strongly interacting with short-term satiety signals.

#### 4.3. Signals associated with body fat

It has been mentioned in Section 1 that energy intake equals energy expenditure so that energy balance is maintained over prolonged periods. The data shown above demonstrate that metabolic and/or gastrointestinal signals associated with nutrient intake are involved in this event. A possibility that has been ignored so far in the present survey is the idea that body weight itself is the regulated factor. A compartment that largely contributes to body weight is the body fat mass and Kennedy postulated several decades ago that the body produces one or more factors proportional to the size of body fat. In turn, these would be signalled to CNS regions involved in regulation of ingestive behaviour and metabolism [5]. Some evidence that these adiposity-related signals are involved in regulation of food intake can, for instance, be observed in the case of chronic involuntary overfeeding. Thus, when the earlier-discussed intragastric infusion of excess nutrients (e.g. twice the daily amount of nutrients as rats would normally ingest) is continued over several days, rats gain weight rapidly and suppress their spontaneous food intake. While the suppression in food intake can be easily explained by the satiety signals associated with the involuntary nutrient load, they hardly if at all explain the suppression of food intake that continues for several days after termination of overfeeding. In the latter instance, the rats continue to display reduced food intake until body weight (read body fat) has returned to the original level. Thus, signals associated with the enlarged body fat stores might play a role in this long-term hypophagia. These mechanisms acting in the opposite direction have been observed after chronic food restriction, i.e. when ad libitum food intake to restricted rats is restored they display increased food intake until body weight has been restored to the original level [52]. The main strategy by which rats reduce their total food intake after involuntary overfeeding, or increase their food intake after food restriction, is through modulation of meal size [52,53].

Evidence that regulation of body fat involves a hormonal feedback signal came from early studies of Hervey and colleagues using parabiotic rats [54]. These are surgically

united pairs of rats which permanently exchange blood through a capillary anastomosis. If one rat is made obese by a lesion in the VMH/arcuate (Arc) hypothalamic region, the other becomes hypophagic and very thin in response to the obesity in the partner, with which it shares a blood supply. The VMH/Arc-lesioned animal, on the other hand, might be regarded as ‘blind’ to the increasing level of the lipostat signal. Over the past several years, many lipostat signals have been proposed, but it took the discovery of leptin that really reinforced the concept of lipostat regulation of body weight and energy balance [55]. Leptin is a protein product of the *OB* gene which is mainly expressed in adipose tissue. Secreted leptin is found in the plasma at levels that are in proportion to stored fat, and it is circulating with a relatively long half-life. Leptin gains access to the central nervous system via a receptor-mediated transport system located in the blood–brain barrier, and acts on receptors that are connected to intracellular signalling cascades (for review see Refs. [56,57]). Alternatively, it could reach certain blood–brain free CNS regions directly. The fact that leptin acts primarily in the hypothalamus to regulate energy balance comes from data showing that administration of relatively low doses of leptin directly administered into the third cerebral ventricle, and even smaller doses in the ventromedial/arcuate region of the hypothalamus, is a particularly potent stimulus to reduce food intake in rats and mice [58]. This area abundantly expresses leptin receptors that are connected to the neuronal circuitry involved in the regulation of food intake [56,57]. Continuation of leptin treatment over several days causes a sustained reduction in food intake and produces a profound loss of body weight and fat mass in the long run [58]. Consistent with the idea that leptin signalling in the CNS closes a feedback loop enabling the level of body fat to be maintained at a certain level, are the observations that leptin deficient *ob/ob* mice [59] and humans [60] (due to a mutation in the *OB* gene) are hyperphagic and obese, and leptin treatment in these individuals is able to normalize ingestive behaviour and aberrated body weight homeostasis [59,60]. Zucker rats and *db/db* mice have a mutation in the leptin receptor gene and are therefore obese as well [61]. It is known that plasma leptin levels fluctuate over the daily cycle, with low levels before the onset of the active (dark phase in rats) and relatively high levels at the end of the active phase [62]. These observations could mean that leptin is involved in the temporal organization of food intake particularly during the onset of the active dark phase in rats. We have investigated the temporal organization of ingestive behaviour of Zucker rats and observed that these animals have a normal feeding pattern comparable to non-mutant rats. The increased food intake in these animals is accomplished by increasing meal size whereas the ratio of light to dark feeding and the temporal distribution of feeding are unaffected [16]. Therefore, the differential food-intake regulation of Zucker rats may not be in the initiation of meals at unusual times in the daily cycle, but in the

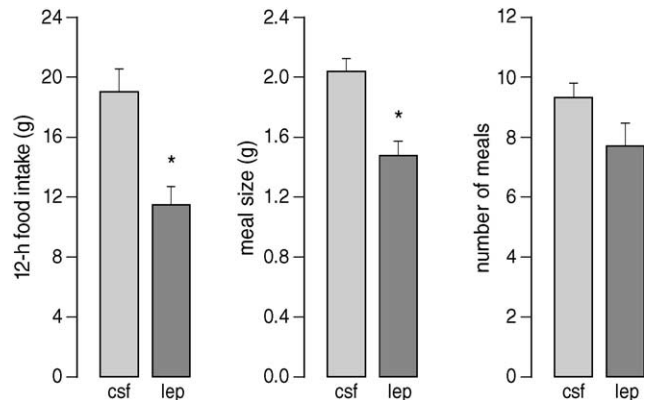


Fig. 7. Effects of intraventricular leptin (lep) on food intake, meal frequency and meal size in comparison with the effects of control infusions of cerebrospinal fluid (csf). From Van Dijk et al. (unpublished observations).

termination of meals. Thus, leptin does not affect the initiation of meals, but rather acts on meal termination and thereby on meal size. We have analyzed the mechanism by which central leptin reduces food intake more carefully. Shortly, ad libitum feeding rats with chronically implanted cannulae in the third cerebral ventricle received leptin (3.0  $\mu$ g) or saline prior to the dark phase. While these animals reduced their overnight food intake by approximately 50%, the main effect by which these animals reduced food intake was through a consistent reduction in meal size without significantly affecting meal frequency (Fig. 7). These data are consistent with those of Flynn et al. [63].

Basal circulating levels of insulin correlate with the body adiposity level as well. Like leptin, administration of low doses of insulin into the cerebral ventricles of rats and baboons produces profound reductions in food intake, and, when infused over days, in body weight as well [64]. These data suggest that insulin is involved in regulation of body weight and energy balance as well. Since the VMH/ARC region of the hypothalamus expresses abundant insulin receptors in addition to leptin receptors, both leptin and insulin might be implicated in the lipostat regulation of energy balance. Indeed, infusion of small amounts of insulin into the VMH/ARC region of the hypothalamus suppresses food intake potently in rats, and infusion of insulin antibodies (presumably immunoneutralizing the ambient insulin) increases food intake and meal size in rats [65]. Like leptin deficiency in rodents and humans, insulin deficiency (due to disruption of pancreatic B cell functioning) is also associated with hyperphagia. Of course, insulin deficient animals are also lean because insulin is necessary for storage of fuels, and the low leptin levels associated with this leanness would be predicted on its own to cause hyperphagia. However, the fact that insulin replacement therapy in the central nervous system of type-I diabetic rats is partly able to reverse the hyperphagia in these animals suggests that insulin action on food intake is independent of leptin [66].

#### 4.4. Interaction of signals related to energetic status

It seems that the mechanism by which the adiposity-related factors are involved in regulation of energy balance are principally different from the ones that are directly associated with nutrient intake. For example, administration of leptin or insulin into the CNS on a chronic basis has dramatic effects on food intake and body weight in the long run. Infusion of the typical satiety hormones CCK and GLP-1 suppress food intake only for a short term, but have no effects on body weight when given on a chronic basis [67, 68]. It has therefore been hypothesized that lipostat signals regulate energy balance on a long term whereas the gastrointestinal and metabolic signals selectively regulate energy balance over a shorter time scale. A number of developments over the last few years suggest a more integrated view.

*Synergism of short- and long-term satiety signals.* One idea postulated previously by Riedy and colleagues suggested that the long-term lipostat might act through the short-term satiety signals. They approached this by infusing a sub-threshold dose of insulin into the third cerebral ventricles of rats and observed that these animals were more sensitive to CCK to reduce food intake [69]. Such interaction was also found between leptin and CCK [70]. The mechanism by which these sort of synergisms act might be such that afferent neural messages conveying gastrointestinal and/or metabolic information are relayed into the neural circuitry that signals changes in leptin and/or insulin. While these interactions of lipostat and afferent neural signals could take place at the level of the hypothalamus [53], they might also occur at lower (brainstem) levels [71].

*Thermogenic effects of leptin.* We [72], and others [73] have previously observed that central leptin administration has profound thermogenic effects, presumably through stimulation of uncoupling proteins (UCP) which are located in several metabolically active tissue including brown adipose tissue (UCP 1), liver and white adipose tissue (UCP2), and muscles (UCP3) (for review see Ref. [74]). These effects of central leptin might be mediated through stimulation of sympathetic outflow to these tissues [73], or alternatively via stimulation of thyroid hormone secretion [75]. Because thermogenesis itself is believed to play a role in determining meal size, any effect of leptin to increase thermogenesis could potentially influence meal size as well [42,72].

*Gastrointestinal effects of leptin.* Besides affecting thermogenesis, leptin also has effects on the gastrointestinal system. For example, 3-day leptin treatment into the third cerebral ventricle of rats causes these animals to reduce their food intake by approximately 50%. Despite this profound reduction in food intake, these animals have normal distension of the gastrointestinal system. This is quite remarkable in light of the finding that animals pair-fed to the leptin treated animals have a robust suppression of gastrointestinal distension relative to the leptin-treated

animals. These data suggest that elevated central leptin levels reduce gastrointestinal transit, and this is consistent with the observation of Smedh et al. who found that central leptin administration reduces gastric emptying [76]. Increased distension of the gastrointestinal tract is usually associated with higher satiety indexes, and this may provide additional evidence that leptin reduces food intake, in part, through its action on peripheral systems (Fig. 7).

#### 5. Concluding remarks and perspectives

The energy content of men and animals is under homeostatic control mediated by the adjustment of food intake. It is discussed in this review that besides signals reporting energy deficits, optimized programs of body clocks take part in feeding behaviour as well. Together, they will set the level of feeding motivation determining when, where and what to eat and what in a certain condition the optimized feeding strategy in terms of meal frequency and meal size will be. These processes are discussed extensively in the different sections of this review and are depicted in Fig. 8.

As discussed in Section 3, the circadian LEO in the SCN is regarded as a reference clock for memory of time and determines for a great deal the timing of meal initiation over the daily cycle, and thus has a major influence on meal frequency. Under certain conditions, a FEO comes into play as well, particularly when large amounts of food are available only at certain (predictable) times of the circadian cycle. This might prepare the body to cope with particularly large meal sizes. Both oscillators determine anticipatory adaptive behavioural and physiological mechanisms, promoting or inhibiting food intake. In fact these clocks form the constraints within which the homeostatic regulation of feeding behaviour must operate. A strong interaction between circadian and homeostatic regulation is evidenced by the fact that meal sizes of rats are not at all correlated to their pre- or postprandial intermeal intervals. Lesioning of

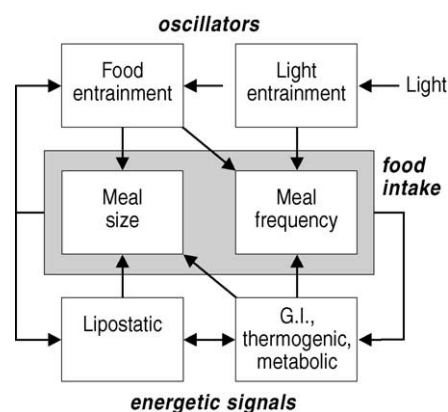


Fig. 8. Summarizing diagram reflecting the temporal organization of food intake in interaction with the regulation of energy balance (GI = gastrointestinal). For description see text.

the SCN reveals a significant postprandial correlation (Table 1) which suggests that initiation of each next meal is a function of the size of the previous one when the influence of the circadian pacemakers is removed and the homeostatic regulation is left intact. In this homeostatic control a wide variety of regulatory negative feedback mechanisms, or satiety signals, play a dominant role. They act in a short duration as well as in the long run, reporting the central nervous system about energy expenditure and/or the current level of energy content of the body. The afferent pathways to the central nervous system may either be nervous or hormonal. As discussed in Section 4.2 several gut hormones such as CCK and GLP-1 function as 'short-term' satiety factors and determine meal sizes and intermeal intervals. Liquid food in the stomach increases the release of these signals and causes a delay of meals except during the last hours of the dark phase when the circadian pacemaker strongly interacts with these satiety signals. This is again a striking demonstration of the strong interacting influence of the circadian clock which determines anticipatory excess feeding behaviour advancing the light phase, i.e. a period when, in nature, the risk of predation would be high. This anticipatory function allows the animal to energetically overcome a period of rest and sleep.

Body temperature may also be an important satiety signal since meals are terminated at almost similar high core temperatures. A major function of food intake, besides directly delivering the necessary fuels for metabolic purposes, is to ensure that energy balance in the long run is maintained on a variety of diets, and thus to maintain body weight within rather narrow limits. In this respect, the adipose tissue mass (which is a major contributor to body weight) may also inform the central nervous system about their content. Among the signals that reflect body weight are the hormones leptin and insulin, and these may be important determinants in the long-term regulation of feeding behaviour. As discussed in Section 4.3 and depicted in Fig. 8, these lipostatic factors (i.e. leptin and insulin) affect meal size directly, or act through interplay with the short-term signals (i.e. gastrointestinal system, and thermogenic and metabolic factors) that are produced during each meal. Thus long-term control of feeding behaviour mainly affects meal termination but not initiation.

In conclusion, the tendency to perform feeding behaviour (hunger) or to stop meals (satiety) at any point in time will depend at least on (1) the current level of energy expenditure; (2) possible shifts in body energy content; (3) the resultant of all satiety signals pooled in the central nervous system; and (4) constraints imposed by circadian light- and food-entrainable oscillators (FEO) and all sorts of learned habits and behavioural interactions. Disturbances of these processes or of their interactions may lead to disorders in feeding behaviour which may result in deviations of body weight such as in obesity. Indeed, obesity in rats is associated not only with deficient leptin or insulin signalling, but also with deficient CCK signalling [77]. The same

may be true for certain abnormalities in thermoregulatory control [78]. A major challenge for the near future is to reveal how these different levels of control are integrated and provide the motivational background for switching a meal in the 'on' and 'off' direction. It is obvious that this is not only a matter of 'how' but also of 'when'.

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