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"Feeding time" for the brain: A matter of clocks

Céline A. Feillet [a], Urs Albrecht [b], Etienne Challet [a,*]

[a] *Department of Neurobiology of Rhythms, Institute of Cellular and Integrative Neurosciences, University Louis Pasteur and CNRS, 67084 Strasbourg, France*

[b] *Department of Medicine, Division of Biochemistry, University of Fribourg, 1700 Fribourg, Switzerland*

Abstract

Circadian clocks are autonomous time-keeping mechanisms that allow living organisms to predict and adapt to environmental rhythms of light, temperature and food availability. At the molecular level, circadian clocks use clock and clock-controlled genes to generate rhythmicity and distribute temporal signals. In mammals, synchronization of the master circadian clock located in the suprachiasmatic nuclei of the hypothalamus is accomplished mainly by light stimuli. Meal time, that can be experimentally modulated by temporal restricted feeding, is a potent synchronizer for peripheral oscillators with no clear synchronizing influence on the suprachiasmatic clock. Furthermore, food-restricted animals are able to predict meal time, as revealed by anticipatory bouts of locomotor activity, body temperature and plasma corticosterone. These food anticipatory rhythms have long been thought to be under the control of a food-entrainable clock (FEC). Analysis of clock mutant mice has highlighted the relevance of some, but not all of the clock genes for food-entrainable clockwork. Mutations of *Clock* or *Per1* do not impair expression of food anticipatory components, suggesting that these clock genes are not essential for food-entrainable oscillations. By contrast, mice mutant for *Npas2* or deficient for *Cry1* and *Cry2* show more or less altered responses to restricted feeding conditions. Moreover, a lack of food anticipation is specifically associated with a mutation of *Per2*, demonstrating the critical involvement of this gene in the anticipation of meal time. The actual location of the FEC is not yet clearly defined. Nevertheless, current knowledge of the putative brain regions involved in food-entrainable oscillations is discussed. We also describe several neurochemical pathways, including orexinergic and noradrenergic, likely to participate in conveying inputs to and outputs from the FEC to control anticipatory processes.

Keywords: Circadian rhythms; Clock gene; Food synchronisation

1. Introduction

As a consequence of the earth's rotation around the sun and around its axis, all living organisms experience cyclic variations of their environment. Circadian clocks are a way of adapting by anticipating these changes. In mammals, the suprachiasmatic nucleus of the hypothalamus (SCN) is the master clock, as demonstrated both by lesion and transplantation studies (Ralph et al., 1990). The SCN is able to elaborate and distribute rhythmic messages of about 24 h to the entire organism (van Esseveldt et al., 2000). These rhythmic signals have been demonstrated to originate in transcriptional/translational feedback loops involving a set of clock genes (Ko and Takahashi, 2006). Among all cues able to synchronise the SCN to exactly 24 h, the light/dark cycle is the most powerful (photic cues). Nevertheless, the SCN clockwork and its photic responses can be modulated by nutritional cues (Castillo et al., 2004; Challet et al., 2003; Lamont et al., 2005; Mendoza et al., 2005b). Moreover, restricted feeding schedules (Mendoza, 2007; Waddington-Lamont et al., 2007) and other non-photic cues, like methamphetamine administration (Iijima et al., 2002), have also been shown to influence behaviour and physiology outside the SCN. Thus, even if the SCN is undoubtedly the master circadian clock, other brain regions and many other cells around the body express clock genes and are capable of sustained clock gene

* Corresponding author. Tel.: +33 3 88 45 66 93; fax: +33 3 88 45 66 54.
E-mail address: challet@neurochem.u-strasbg.fr (E. Challet).

oscillations (Welsh et al., 2004; Yoo et al., 2004; Nagoshi et al., 2005). Does this generalized clock gene expression suggest a network of local oscillators commanded by the master SCN, or does it reveal the existence of new central clocks? Considering recent data, we would favour the second possibility.

2. Existence of another clock: the food entrainable clock (FEC)

2.1. The first hints for the existence of the FEC

In 1979, F.K. Stephan and colleagues demonstrated that in rats made arrhythmic by bilateral lesion of the SCN, a circadian bout of locomotor activity can be restored when the animals are submitted to a restricted feeding schedule, i.e. an access to food limited to a certain duration, delivered every day at the same time point. In these conditions, the animals start to run in the hours prior to food access, thus restoring circadian locomotor activity (the so-called the Food Anticipatory Activity: FAA; Stephan et al., 1979). This phenomenon is not limited to activity but is also observed in core body temperature, which rises in the hours preceding food access (Food Anticipatory Thermogenesis), and in plasma corticosterone, which peaks during Food Anticipatory Corticosterone (Mistlberger, 1994). Interestingly, the FAA can be entrained only if food is presented within the circadian range (between 22.5 and 29 h; Stephan and Becker, 1989). Moreover, when food access is shifted, the FAA is progressively shifted to the new feeding time (Stephan, 2002). In addition, it has been established that the FAA is also expressed in animals with an intact SCN housed in constant darkness (DD, e.g. Mistlberger, 1994), or in LD when food is presented during the inactive phase (Castillo et al., 2004; Mendoza et al., 2005b).

All of these clues seem to indicate the existence of another clock, distinct from the SCN and entrained by food: the Food Entrainable Clock (FEC). This clock would have multiple outputs, among which one finds activity (FAA), temperature and corticosterone. Under ad libitum feeding conditions, these outputs would be phase locked to the SCN clock. Under restricted feeding conditions, daily patterns of activity, temperature and plasma corticosterone would result from the influence of both clocks.

2.2. How and where is the FEC ticking?

2.2.1. Functioning of the SCN

It appears that clock mechanisms share common features in all organisms investigated from cyanobacteria to mammals (Wijnen and Young, 2006). They all rely on transcriptional/translational feedback loops involving the so-called "clock genes". In the past few years, we have learned more and more about the SCN clockwork, and all cellular clocks in mammals seem to work in roughly the same way.

It is now known that circadian oscillations rely on two positive and negative feedback loops. In the main positive loop, two genes *Clock* and *Bmal1* (members of the bHLH-PAS family) are transcribed and translated into proteins that dimerize and activate the transcription of *Period (Per) 1-2-3*, *Cryptochrome (Cry) 1-2*, *Rev-erbα*, *rorα* genes via E-Box sequences in their promotors (Albrecht and Eichele, 2003; Okamura et al., 2002; Shearman et al., 2000). PER-CRY heterodimers inhibit their own transcription by interacting with the BMAL1/CLOCK dimers (main negative loop) while REV-ERBα directly inhibits Bmal1 transcription (secondary negative feedback loop, Preitner et al., 2002). RORα acts in the opposite way (secondary positive feedback loop, Akashi and Takumi, 2005; Sato et al., 2004). The CLOCK/BMAL1 dimer is also able to activate the transcription of "clock-controlled genes" (CCG) by interacting with E-Boxes in their promoters. CCG then deliver a rhythmic output to control physiology and behaviour. Among those CCGs, vasopressin and albumin D-site binding protein (DBP) are often taken as phase reference of the SCN (Ko and Takahashi, 2006).

2.2.2. Does FEC function rely on the same genes? The mutant approach

To assess whether the FEC clockwork relies on the same genes as the SCN, mutant mice for diverse clock genes have been challenged with restricted feeding schedules, hypothesizing that FAA should be expressed if the mutation does not affect FEC functioning; conversely, mutation of a gene that is critical for the FEC should impair FAA expression.

The first gene to be tested in this respect was a member of the positive feedback loop, the *Clock* gene (Pitts et al., 2003). Under regular LD conditions and ad libitum feeding, *Clock* mutants exhibit normal entrainment, but in constant darkness their free running period is lengthened drastically compared to wild-type mice and they become mostly arrhythmic (Vitaterna et al., 1994); note that a single nucleotide transversion in a splice donor site, causing a deletion in the transactivation domain of the CLOCK protein is responsible for this phenotype (King et al., 1997). Given that CLOCK is important for time-keeping in the SCN, it was expected to be involved in food synchronisation. When submitted to a daily 4 h food access in LD and in DD conditions, *Clk*/*Clk* mutant mice exhibit FAA, even when their circadian wheel-running behaviour is arrhythmic in DD conditions (Pitts et al., 2003). Apparently, the *Clock* gene is not a part of the FEC clockwork. It is noteworthy that *Clock* KO mice have been recently generated (Debruyne et al., 2006). Unlike the *Clock* mutant mice, the KO mice remain rhythmic in DD and free-run with a period similar to WT mice. This result raises the possibility that the *Clock* gene would not be as essential as postulated based on the *Clock* mutant mice phenotype. This finding also questions the results found for the FEC functioning. Until that question is addressed, we would state that the *Clock* gene is not essential for the FEC clockwork.

In 2001, another clock gene was identified as an analogue of *Clock*:*Npas2* (Reick et al., 2001). It dimerizes with BMALL and is expressed throughout the forebrain. It was suggested to replace CLOCK in its functions outside the SCN. To investigate the possible role of NPAS2 in behavioural manifestations of circadian rhythm, Dudley and colleagues studied locomotor activity and sleep patterns under restricted feeding conditions in *Npas2*-deficient mice (Dudley et al., 2003). When fed ad libitum, locomotor activity in *Npas2*−/− mice is unaffected. In DD, they are perfectly rhythmic with a period slightly shorter than their wild type littermates. Even if their pattern of activity is somewhat different from that of wild-type mice, considering that NPAS2 appears not to be expressed in the SCN, no major alteration in circadian behaviour was to be expected. Furthermore *Npas2*−/− mice were placed under restricted feeding schedules to measure FAA: the results indicate that even if FAA does not disappear in these KO mice, its expression is delayed, indicating that NPAS2 plays a role in adaptability to food restriction. So there may be a slight modification of the molecular feedback loops in the FEC compared to the SCN, including a replacement of CLOCK by its analogue NPAS2.

As mentioned above, the *Cry* genes are essential components of the SCN clockwork, belonging to the main negative feedback loop. The involvement of *Cry* genes has been tested in food anticipatory behaviours (Iijima et al., 2005). If *Cry1* or *Cry2* single mutant mice do not show major defects in circadian behaviour, *Cry1*/*Cry2* double mutants become arrhythmic upon transfer to DD when fed ad libitum. Their persistent rhythmicity in LD has been interpreted as a masking effect by light (Van der Horst et al., 1999; Vitaterna et al., 1999). Compared to wild-type littermates, food-restricted *Cry1*−/−/*Cry2*−/− mice expressed a FAA that was less stable and with delayed onset (Iijima et al., 2005). So it seems that the *Cry* genes are not essential for synchronisation to feeding schedules, but rather affect the stability and development of the FAA, being a part of the FEC.

All these studies pointed to modest alterations in the FAA rather than drastic suppression of the FEC output. It is possible that the outputs chosen did not reflect actual defects in the FEC, and that other outputs like corticosterone may have given clear-cut results.

The *Per1* gene, originally cloned by homology to *dPer* in Drosophila (Tei et al., 1997), and the *Per2* gene (an homologue of *Per1*) have been demonstrated to be critical for normal synchronisation of the SCN to light (Albrecht et al., 1997, 2001; Shigeyoshi et al., 1997; Spoelstra et al., 2004). With regard to their role in the SCN clock, it seems that not all *Per* genes are equal: normal under LD conditions, Per1 KO mice are still rhythmic in DD (Cermakian et al., 2001; Zheng et al., 2001) (although another line of *Per1* mutant mice become arrhythmic in similar constant conditions: Bae et al., 2001). Two lines of *Per2* mutant mice become arrhythmic after a few days in DD (Bae et al., 2001; Zheng et al., 1999). Being critical for SCN synchronisation to light, it was possible that *Per* genes would also be responsive to other kinds of synchronizers, e.g. food. We challenged both *Per1* and *Per2* mutant mice with restricted feeding in LD and showed that there is no significant alteration in FAA expression in *Per1* mutant mice (Feillet et al., 2006). Interestingly, *Per2* mutant mice failed to show any anticipation of meal time as assessed by wheel running and general cage activity, whether in LD, DD or LL conditions for three weeks under a temporal restricted feeding schedule, or if only a daily hypocaloric diet is given. In this latter case, mice are provided 66% of their normocaloric intake, given daily at a fixed time point, measured as the average daily intake over 2 weeks of ad libitum feeding. This protocol is known to exert a very powerful effect on the SCN clock whereas restricted feeding alone does not (Mendoza et al., 2005b). Moreover, mice lose about 20% of their initial weight under hypocaloric feeding, as opposed to traditional restricted feeding, where mice usually maintain their weight. Added to the lack of anticipation of meal time in wheel running and general cage activity, the anticipatory bout of temperature rise was also absent in food-restricted *Per2* mutant mice, reinforcing the hypothesis that they demonstrate major impairment of the FEC, as shown by the alteration of two of its outputs (Feillet et al., 2006). These results therefore suggest a critical role of *Per2* but not *Per1* in the molecular regulation of the FEC. Even if not all the clock gene mutants have been challenged with limited access to food, we can nonetheless propose a putative model for the FEC clock, mirroring to some extent that of the SCN (Fig. 1). Afferent and efferent pathways, sensors to and effectors of the FEC have nonetheless yet to be clearly identified, as well as its exact location.

2.3. Location of the FEC

Many hypotheses have been raised concerning the anatomical substrate of the FEC, whether it be a single central structure, a single peripheral organ, or a multi-oscillator system within the central nervous system. Because many peripheral organs such as intestine, kidney or liver express clock genes and can be entrained by restricted feeding schedules (Damiola et al., 2000; Hara et al., 2001; Stokkan et al., 2001), it has been proposed that they might actually be a part of the food-entrainable clock, or constitute the FEC itself.

Experiments on rats made cirrhotic by CCl_4 injection show that the FAA is not abolished in these animals, even though hepatic function is severely altered (Escobar et al., 2002). In addition, in food-restricted *Per1* luciferase rats, FAA does not arise as an output of rhythms in the gastrointestinal system, suggesting that the FEC does not reside in the liver or in other peripheral tissues of the digestive system (Davidson et al., 2003). These organs probably contain outputs of the FEC, or even sensors which indicate the metabolic state of the body to the brain, but the FEC may arise as a central structure or network of structures, that

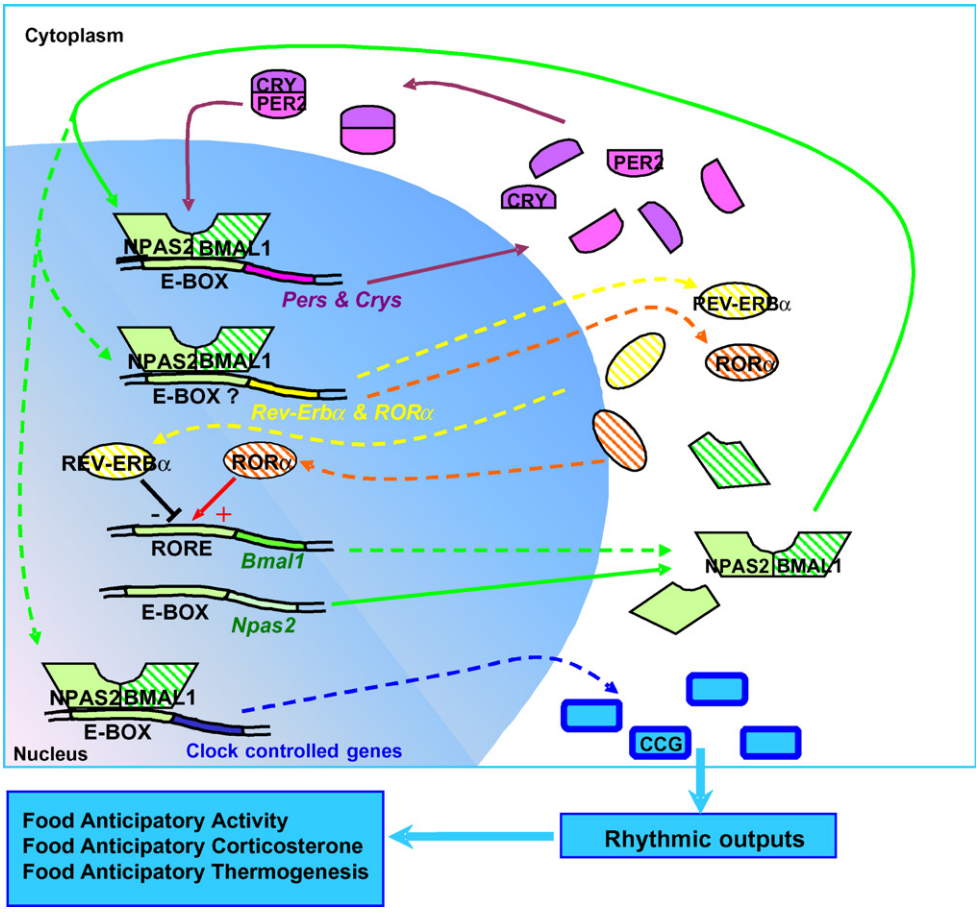

Fig. 1. Putative clockwork of the Food-entrainable Clock (FEC). This scheme represents genes possibly implicated in FEC functioning. So far *Npas2*, *Cry* and *Per2* have been clearly demonstrated to participate in this mechanism. Therefore their proteins and connected arrows are in plain colours. Other genes and proteins shown here, the roles of which are inferred from known mechanisms occurring in the SCN, are represented with hatched colours and dashed arrows. Their involvement in the FEC clock is still speculative and remains to be demonstrated.

receives and integrates information from the periphery, and in turn generates a new temporal message that is distributed to all peripheral organs. It is noteworthy that neither *Per1* nor *Per2* appear to be critically involved in the synchronisation of peripheral organs to meal time (Feillet et al., 2006).

Numerous experiments have been performed to locate this new clock. Speculating it to exist as a single structure in the central nervous system, lesions of the hippocampus (Mistlberger and Mumby, 1992), the neocortex (Mistlberger, 1994), the ventromedial nucleus of the hypothalamus (Mistlberger and Rechtschaffen, 1984), the lateral area and paraventricular nucleus of the hypothalamus (Mistlberger and Rusak, 1988), the arcuate nucleus of the hypothalamus (Mistlberger and Antle, 1999), the area postrema (Davidson et al., 2001a), and the olfactory bulbs (Davidson et al., 2001), have had little if any influence on FAA expression. Interestingly, while complete ablation of the nucleus accumbens does not impair FAA (Mistlberger and Mumby, 1992), specific damage of the core but not the shell reduces the expression of FAA (Mendoza et al., 2005a).

Lesions of the parabrachial nucleus alter both FAA and food anticipatory thermogenesis, but the authors concluded that this structure is a relay for information to or from the FEC rather than constituting the FEC itself (Davidson et al., 2000). Another publication showed a dissociation between FAA and food anticipatory thermogenesis in SCN-lesioned and hypophysectomised rats: the anticipatory rise in temperature was no longer expressed under restricted feeding conditions, whereas FAA was still present. Although this study did not unravel the location of the FEC, it demonstrated the possibility to distinguish different outputs of a same clock (Davidson and Stephan, 1999). This kind of dissociation between food anticipatory variables has also been noted after chemical lesions of the infralimbic cortex, that impair specifically food entrainable thermogenesis, but not FAA (Recabarren et al., 2005).

Lesions of the dorsomedial hypothalamus (DMH) have been shown recently to markedly impair FAA expression (Gooley et al., 2006). In addition, it was demonstrated that *Per1* expression becomes rhythmic in this structure only under restricted feeding (Mieda et al., 2006). In spite of the fact that the DMH may participate in the FEC,

chemical lesions of the DMH also clearly diminish baseline activity (Gooley et al., 2006). Moreover, another recent study showed that FAA persists after electrolytic lesion of the DMH (Landry et al., 2006). Transplantation studies as well as rescue of function will provide more definitive arguments to delineate the actual role of the DMH with respect to food synchronisation.

In light of the contradictory data obtained when lesioning a single central structure, an increasing number of studies suggest that the FEC may result from the combined activity of a network of structures with partially redundant function. This hypothesis was exemplified in studies that measured sustained c-FOS expression in anticipation of meal time in hypothalamic regions like the lateral hypothalamus, perifornical area and DMH, that persisted when food was not provided at the expected time (Angeles-Castellanos et al., 2004). In food entrained rats, c-FOS immunoreactivity was also observed in anticipation to and after meal time in nucleus accumbens, basolateral and central amygdala, in the bed nucleus of the stria terminalis, lateral septum, prefrontal cortex and paraventricular nucleus of the thalamus, but not in the hippocampus (Angeles-Castellanos et al., 2007). This study thus shows the possible involvement of cortico-limbic structures in food synchronisation.

The brainstem receives all the information from the gastrointestinal system. Considering the importance of communication between peripheral organs and central structures, it was of interest to characterize the response of the brain stem to food restriction. It was demonstrated that the expression of c-FOS immunoreactivity was increased after meal time in a number of brainstem nuclei. These increases were no longer detectable if food was not presented at the expected time (Angeles-Castellanos et al., 2005). This work reveals the probable involvement of brainstem structures in conveying food ingestion information to the brain, but not directly in FEC functioning.

Another approach in searching for a network underlying the FEC came from the study of modifications of local cerebral metabolic rate for glucose in response to food restriction at the time of FAA. Glucose utilization was assessed by the 2-deoxyglucose technique in various brain areas in food-restricted rats compared to animals fed ad libitum. It was hypothesized that structures undergoing major changes in their glucose consumption in response to food restriction would play a role in the network underlying the FEC, or at least in FAA expression. The data showed specifically diminished glucose consumption in the intergeniculate leaflets, the paraventricular hypothalamic and thalamic nuclei, the medial preoptic area, the arcuate nucleus, the nucleus of the solitary tract, and the cerebellar cortex in food-restricted rats compared with control animals (Pereira de Vasconcelos et al., 2006). Taken together, these studies show the complexity of food entrainment and support the hypothesis that the FEC may not reside in a single brain structure. If it is actually a network of structures, it would explain why single lesion studies failed to localise the site hosting the FEC. Unfortunately, neither c-FOS immunoreactivity nor local glucose utilization address a possible hierarchy in these structures: is there among them a conductor giving tempo to the others, or do they all participate in food-entrained rhythms? This question is still open, and genesis of food-entrainable oscillations will have to be addressed in the coming years.

2.4. Neurochemical pathways involved in the expression of FAA

2.4.1. Glutamatergic, histaminergic and opioidergic pathways

Among the neurochemical systems involved in FAA is the glutamatergic network. Blockade of NMDA receptors reduces the expression of FAA in a dose-dependent manner (Ono et al., 1996). However, this interesting result does not provide specific clues to locate structures involved directly in the FEC because NMDA receptors are so widely expressed in the central nervous system.

Brain histamine is considered to participate in the regulation of feeding behavior. Of interest, histaminergic neurons of the tuberomammillary nucleus have been shown to be activated during FAA (Meynard et al., 2005). Further studies will be helpful to determine whether hypothalamic histaminergic neurons are situated on the afferent or efferent pathways of the FEC network.

The mesolimbic opioid-dopamine system plays a key role in motivated behaviours. Mu-opioid receptor KO mice challenged by restricted feeding display reduced FAA compared to WT, while their dopaminergic system remains unaffected (Kas et al., 2004). These findings therefore indicate that the opioidergic system plays a role in the regulation of FAA.

2.4.2. The orexinergic pathway, an efferent of the FEC promoting FAA

The orexinergic system has also been considered with respect to food anticipation. Orexins (hypocretins) A and B are neuropeptides whose receptors (orexin-1 and orexin-2, receptors) are found mainly in the forebrain, in hypothalamic, thalamic and brainstem nuclei and in spinal cord. Orexinergic neurons are located only in the lateral/perifornical hypothalamic area. They promote locomotor activity, food intake and motivated behaviours, and coordinate sleep/wake patterns. Mice lacking either the orexin gene or orexinergic neurons show phenotypes similar to human narcolepsy. Moreover, central administration of orexin triggers feeding behavior (Mignot et al., 2002). Considering the importance of orexins in the coordination of wakefulness and motivated behaviours like food seeking, Akiyama and colleagues tested activation of orexin neurons (c-FOS immunoreactivity) under RF, and FAA in wild type and orexinergic neuron-ablated mice. Orexinergic neuronal activation is advanced by 6 h in wild type mice in response to daytime RF. Interestingly, FAA is reduced in orexinergic neuron-ablated mice under the same

conditions. It seems that orexinergic neurons would not generate FAA per se, but they would participate in the wakefulness component of FAA (Akiyama et al., 2004). These results were confirmed by another group that studied the involvement of orexin in the establishment and maintenance of FAA induced by restricted feeding (Mieda et al., 2004). Interestingly, activity of orexinergic neurons markedly increases during food anticipation under restricted feeding in wild-type mice (Mieda et al., 2004). Taken together, these studies would suggest that orexin neurons may convey an efferent signal from the FEC, thus increasing wakefulness, and promoting and maintaining FAA in the hours prior to food.

In natural conditions, these neurons may be essential for the animals to express a seeking behaviour in response to a reduction in food availability. However, they would not be the site of the FEC, because neither electrolytic lesions of the lateral hypothalamic area (Mistlberger and Rusak, 1988), nor targeted ablation of orexinergic neurons (Mistlberger et al., 2003) abolish FAA. It is also possible that orexinergic neurons specifically control locomotor output of the FEC. Considering that no data exists on temperature and corticosterone secretion, these two outputs of the FEC may be expressed normally in such mutants. That question remains to be addressed.

2.4.3. Noradrenergic projections as possible routes from or to the FEC

Ear2 (Nr2f6), COUP-TFI and COUP-TFII are members of a subfamily of orphan receptors involved in the formation of midbrain dopaminergic neurons. Ear2 can homo- or hetero-dimerize with COUP-TFI and COUP-TFII (Avram et al., 1999) and bind to enhancers in the sequence of various genes. *Ear2*−/− mice lack the major part of the locus coeruleus, which provides the majority of noradrenergic transmission in mammals (Warnecke et al., 2005) and contacts a great number of brain structures, particularly in the forebrain. Starting with the observation that *Per* gene expression is dampened in the frontal cortex of *Ear2*−/− mice, it was hypothesized that these mice have a defective circadian timing system in the forebrain. When *Ear2*−/− mice are challenged with restricted feeding, FAA is significantly reduced, concomitant with a drastic reduction of noradrenaline concentration in the frontal cortex (Warnecke et al., 2005). Though the reduction of FAA is obvious in this experiment, noradrenalin

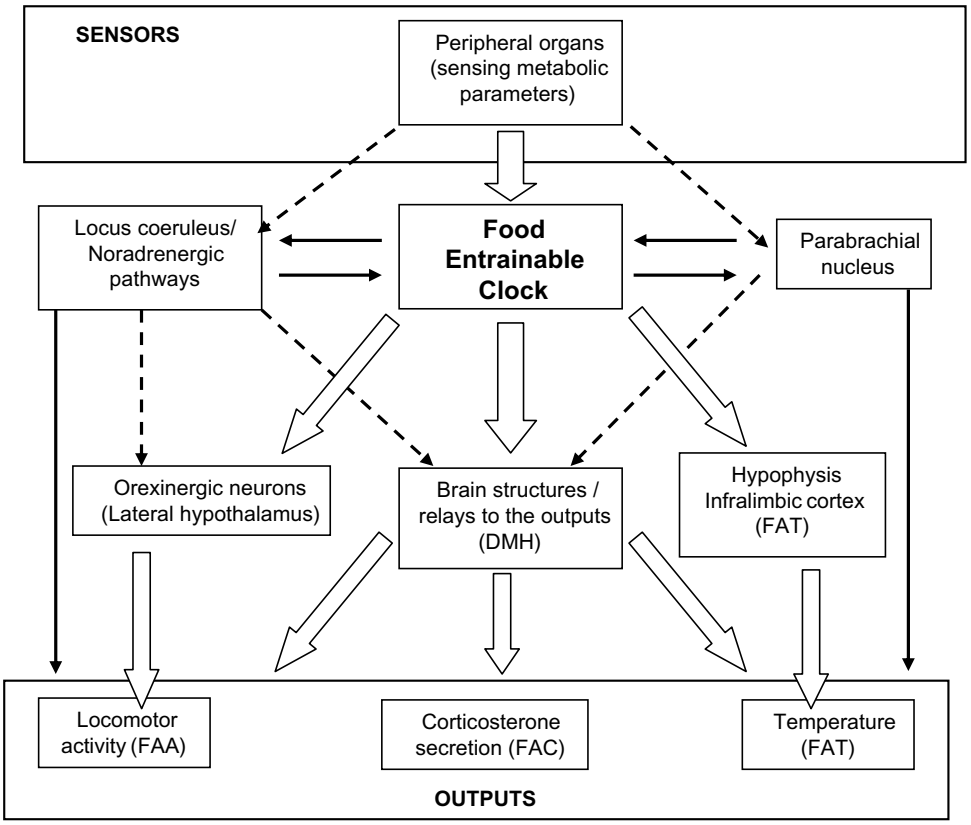

Fig. 2. A model for the Food-entrainable Clock (FEC). This scheme represents the possible network underlying mechanisms related to food synchronisation. Plain open arrows represent demonstrated mechanisms. Plain-line and dashed-line arrows represent probable and more hypothetical pathways, respectively. A central FEC receives information from peripheral organs such as the liver, stomach or intestine. Possible relays (locus coeruleus, parabrachial nucleus) are also connected to the periphery and may deliver redundant messages to the FEC. They can also serve as relay outputs from the FEC. Outputs originating from the FEC are distributed to brain structures that in turn control behavioural and physiological anticipatory outputs. DMH: dorsomedial hypothalamic nuclei, FAA: food anticipatory activity, FAC: food anticipatory corticosterone secretion, FAT: food anticipatory thermogenesis.

may not be the unique transmitter: neurons in the locus coeruleus also express neuropeptides such as vasopressin, galanin and neuropeptide Y, which may be responsible for the observed reduction in frontal cortex rhythmicity and indirectly related to reduced FAA. Moreover no expression of known clock genes could be detected in the locus coeruleus (Warnecke et al., 2005), which would indicate that this structure does not harbour its own clock but constitutes a relay conveying information to the forebrain, possibly from or to the FEC. If this were the case, functional impairment of the locus coeruleus could lead to only partial delivery of inputs to the FEC or incomplete output signals to its effectors, thus resulting in reduction of FAA.

2.5. A possible model for the FEC network

Although the increasing number of studies on the FEC adds complexity to the scheme, they provide new information that allows us to propose a model for the FEC network (Fig. 2).

3. Conclusion

After 30 years of research on the FEC, only the physiological and behavioural outputs of this clock seem to be clearly defined, such as locomotor activity, temperature and corticosterone. As for the FEC itself, it remains a mystery: its exact location, afferent and efferent pathways are still not fully defined. Nevertheless, it seems that the DMH represents a good candidate to be involved in this mechanism, and will certainly be targeted in future work. Orexinergic neurons could provide an output pathway from the FEC, and noradrenergic connexions are probably a part of the FEC network. Recently, much effort has been made to unravel the clockwork that governs the FEC oscillations: especially *Per2*, and to a less critical extent *Npas2* and *Cry* genes, have been implicated in the FEC machinery. Other molecular actors known for their involvement in the SCN have yet to be tested regarding the FEC question.

We should keep in mind that discovering the in and outs of the FEC will open doors for the treatment of feeding rhythm disorders, such as those experienced by shift workers or patients suffering from night-eating syndrome. Their erratic feeding rhythms often lead to obesity, digestive and cardiovascular problems. Hopefully, appropriate synchronisation of feeding rhythms in these patients will alleviate those symptoms.

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