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Prefrontal Cortex and Dorsomedial Hypothalamus Mediate Food Reward-Induced Effects via *npas2* and *egr1* Expression in Rat

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Summary

The effects of food reward on circadian system function were investigated in the hypothalamic nuclei, prefrontal cortex and liver. Food rewards of small hedonic and caloric value were provided for 16 days 3 h after light phase onset to male Wistar rats. The daily pattern of locomotor activity was monitored. Gene expression profiling performed in the dorsomedial hypothalamus (DMH) and liver at the time of reward delivery indicated transcriptional factors *egr1* and *npas2* as possible mediators of food reward effects. Candidate genes were measured in the suprachiasmatic nuclei (SCN), DMH, arcuate nucleus (ARC), prefrontal cortex (PFC) and liver along with *per2* expression. A daily pattern in glycemia and *per2* expression in the SCN was emphasized by food reward. The expression of *egr1* was rhythmic in the SCN, DMH, PFC and liver and food reward weakened or diminished this rhythm. The expression of *npas2* was rhythmic in all tissues except for the PFC where food reward induced rhythm in *npas2* expression. Food reward induced *npas2* and *egr1* expression in the DMH at the time of reward delivery. We suppose that the DMH and PFC participate in the adjustment of the circadian system to utilize food reward-induced input *via egr1* and *npas2* expression.

Key words

npas2 • *egr1* • DMH • Prefrontal cortex

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Introduction

A variety of interfering signals have influenced the activity of the circadian system in recent industrial society. In vulnerable individuals, impaired circadian system function can facilitate the development of pathologies. Recently this problem has attracted attention and strategies on how to achieve a proper synchronization of susceptible parts of the population are emerging. Although the role of melatonin, light control, sleep conditions and food intake in resynchronization is undisputable (Skene and Arendt 2006), there is still a problem with long-time acceptance of these treatments. Therefore, we focused on food reward as a possible alternative and/or supplemental tool for new and/or existing protocols.

The circadian system is hierarchically organized with the central oscillator localized in the hypothalamic suprachiasmatic nuclei (SCN) and peripheral oscillators localized in all other tissues. The molecular basis of the circadian oscillator relies on a feedback loop created by clock genes. There are three homologues of the *per* gene (*per1*, *per2* and *per3*) and two homologues of the *cry* gene (*cry1* and *cry2*) in mammals. Their expression is induced *via* the E-box regulatory region by a heterodimer formed by the transcriptional factors CLOCK and BMAL1 (Damiola *et al.* 2000). Transcription factor NPAS2 (Neuronal PAS domain-containing protein 2) can functionally replace CLOCK (Reick *et al.* 2001).

A crucial role of clock genes in physiology is

supported by experiments showing that *bmal1* and *clock* deficiency is associated with metabolic disturbances, *per1* and *per2* knockout is linked to changed cancer susceptibility and mutations in *cry1/2* cause altered sleep pattern (Ko *et al.* 2006). The role of NPAS2 was investigated mainly in the forebrain. *npas2*-/-deficient mice exhibit modified behavior, react more rapidly to a changed light (L) dark (D) cycle than control and show restricted food-driven entrainment (Dudley *et al.* 2003).

The ways in which central and peripheral oscillators are synchronized differ (Damiola *et al.* 2000, Monošíková *et al.* 2007). The central oscillator is entrained mainly by the LD cycle and peripheral oscillators are very sensitive to food intake as a synchronizing cue (Damiola *et al.* 2000). Regulation of peripheral oscillators by food reward was investigated predominantly by evaluating changes in clock gene expression in the brain tissues. A rhythmic pattern in *per2* expression was reported in the basolateral amygdala, oval nucleus of the bed nucleus of the stria terminalis, central nucleus of the amygdala and dentate gyrus without an effect of food reward on *per2* expression (Verwey *et al.* 2007). Food reward influences the expression of *per1* in the SCN but much more pronounced effects were observed in the striatum, cingulated cortex and piriform cortex (Mendoza *et al.* 2010).

A food reward can be interpreted by its hedonic as well as caloric value and there are many brain structures that can transmit this stimulus to the circadian system. The lateral hypothalamic nuclei and arcuate nucleus (ARC) are known to play a key role in food intake control (Lenard and Berthoud 2008). The dorsomedial hypothalamic nucleus (DMH) participates in food entrainment (Tahara and Shibata 2013), circadian regulation of arousal and wakefulness and responsiveness to the environmental cues important to animals (Schwartz and Roth 2008). The DMH is bidirectionally interconnected with the SCN (Morin 2013), which serves preferentially to transfer SCN regulation to the periphery but can also mediate DMH influence on the central oscillator. The prefrontal cortex (PFC) receives afferents from the thalamic nuclei and limbic structures; it is involved in many complex cognitive operations, in spatial and visual working memory, and is critical in executive functions involved in choosing behavioral strategy (Webb *et al.* 2015).

Food reward was shown to possess the capacity to synchronize the circadian system, although in the presence of the LD cycle its effect is overwhelmed by the light entrainment (Mendoza *et al.* 2010, Challet and Mendoza 2010). The influence of food reward on the circadian system probably depends on the hedonic value of the reward. In the case of a highly attractive reward or addictive drug, the mesocorticolimbic system is activated (Challet and Mendoza 2010). To eliminate the risk of mesocorticolimbic system overactivation and deregulation of energy homeostasis, a food reward of low hedonic and caloric value was chosen in our experiment.

The aim of the present study was to identify genes involved in food reward-induced processes in the SCN, DMH, ACR, PFC and liver after the selection of candidate genes by gene expression profiling. Food rewards of small hedonic and caloric value were applied during the light time. Candidate genes were analyzed along with *per2* expression. To describe the function of the central oscillator a daily pattern of locomotor activity and glucose levels in plasma were monitored. The effect of food reward was compared to the effect of food restriction.

Methods

Effect of food reward – Experiment 1

Male Wistar rats (n=86) were obtained from VELAZ Praha (Czech Republic) at the age of 10-11 weeks. The animals were exposed to a 12:12 LD cycle with food and water *ad libitum*. After an acclimatization period the control group was exposed to the same regimen until the end of the experiment. The second group was provided with a food reward (0.4 g piece of sponge biscuit) at ZT3 (ZT – Zeitgeber time, relativized time units; ZT0 corresponds to the beginning of the light phase of the LD regimen). The effect of the food reward was tested after 16 days of administration over a 24 h period in 4 h intervals. Samples of liver, blood and whole brains were taken at ZT2, ZT4, ZT6, ZT10, ZT14, ZT18 and ZT22 as described previously (Herichová *et al.* 2013).

Effect of food reward and food restriction – Experiment 2

Male Wistar rats (n=90) were obtained from Dobra Voda (Slovak Republic) at the age of 9-10 weeks. The animals were treated under the same conditions as in Experiment 1. The effect of food reward combined with food restriction was tested after 16 days. A food reward was provided at ZT3 together with standard pelleted food available for two hours (ZT3-ZT5). Sampling was performed as in Experiment 1.

The experimental protocols were approved by the Ethical Committee for the Care and Use of Laboratory Animals at Comenius University Bratislava and the State Veterinary Authority. Experiments were carried out in accordance with the 86/609/EEC.

Glucose levels were measured in 10 µl of plasma using commercial kits (GLU L1000, Erba Lachema s.r.o., Czech Republic).

The total RNA from tissues was isolated and gene expression was measured as described previously (Herichová *et al.* 2013). To identify genes involved in food reward response, the RT^2 Profiler^{TM} PCR Array "Rat Circadian Rhythms" (PARN-153C) (Qiagen, Germany) was used. cDNA from the DMH and liver sampled at ZT4 (1 h after food reward) from Experiment 1 and cDNA from the liver sampled at ZT2 (1 h before food administration) from Experiment 2 were used to evaluate the effect of treatment on gene expression. We used samples taken at the time of the strongest behavioral response to the treatment in both experiments. Up- and downregulated genes were identified for each treatment (Table 1) and the expression of *egr1* and *npas2* was measured in all tissues along with that of *per2*.

Locomotor activity was monitored throughout Experiments 1 and 2 in home cages in the animal facility as described previously (Herichová *et al.* 2013).

Statistical evaluation

Daily profiles of gene expression and glucose were fitted into a cosine curve with a 24 h period and when experimental data significantly matched the cosine curve its parameters were calculated with 95 % confidence limits. The goodness of fit (R value – correlation coefficient) of the approximated curve was estimated by analysis of variance (ANOVA) (Herichová *et al.* 2013, Monošíková *et al.* 2007). Time is expressed in relative units – Zeitgeber time (ZT), where ZT0 is defined as the beginning of the light phase of the 24 h cycle. An unpaired t-test was used to test differences between two groups. Three groups were compared by one-way ANOVA. Data in graphs are presented as arithmetic mean and standard error of the mean (SEM).

Results

By day 16 of reward providing its consumption by animals is a well-trained habit. Food reward did not induce an anticipatory behavior as it was observed in food-restricted rats. However, we observed a significant increase in locomotor activity during the first hour after food reward administration ($P<0.01$, t-test). The averaged activity during the light part of the LD cycle did not differ significantly between the control and rewarded groups (t-test). Both the control and the rewarded group showed a typical daily pattern of locomotor activity with high levels during the dark phase. Food reward did not influence the amount of locomotor activity during the dark time (data not shown).

The averaged dark time values of plasma glucose levels showed an increase compared to light time concentrations (t-test, $P<0.05$) in the control group, although the cosinor did not confirm a rhythmic pattern. Administration of food rewards over 16 days caused an increase in the robustness of the rhythmic pattern of glucose levels with the maximum during the dark part of the 24 h cycle (cosinor, Table 2).

Administration of food reward influenced the expression of eight genes with fold change >2 in the DMH. The most pronounced increase was observed in *npas2* expression (Table 1).

We observed an increase in *egr1* mRNA in the liver one hour after food reward (1.91-fold change). However, food reward did not show a strong impact on gene expression in the liver (Table 1).

Food restriction caused more than a twofold change in the expression of 19 genes in the liver. The most pronounced changes were observed in clock gene expression due to huge phase shifts induced by food restriction (Table 1).

Food reward was a much more efficient regulator of gene expression in the DMH than in the liver. Food reward combined with food restriction was a much more efficient input than food reward alone in the liver.

We observed a significant increase in *npas2* and *egr1* expression in the DMH of rats with access to food rewards over 16 days at ZT4 compared to the levels observed at ZT2 and ZT6 (Fig. 1A). The increase in *npas2* and *egr1* at ZT4 caused a diminishing of the daily rhythm in *npas2* and *egr1* expression in the DMH (Fig. 1B, cosinor, Table 2). The expression of *per2* showed a rhythmic pattern with a peak at the beginning of the dark part of the LD cycle in the DMH and goodness of fit of the rhythm numerically increased in the rewarded group of rats (Fig. 1B).

Table 1. Effect of food reward and food restriction on gene expression in the DMH and liver of rats synchronized to a 12:12 LD cycle.

Effect of food reward on gene expression in the DMH			
Fold increase compared to control			
2.40	**Npas2**	**Neuronal PAS domain protein 2**	NM_001108214
2.27	Nkx2-5	NK2 transcription factor related, locus 5 (Drosophila)	NM_053651
1.41	Timeless	Timeless homolog (Drosophila)	NM_031340
1.29	Opn3	Opsin3	NM_001191933
1.21	Hlf	Hepatic leukemia factor	XP_003752414
Fold increase compared to control			
-2.15	Atoh7	Atonal homolog 7 (Drosophila)	NM_001170482
-2.16	Mtnr1a	Melatonin receptor 1A	NM_053676
-2.71	Prf1	Perforin 1 (pore forming protein)	NM_017330
-3.47	Epo	Erythropoietin	NM_017001
-5.40	Tcfap2a	Transcription factor AP-2, alpha	NM_001107345
Effect of food reward on gene expression in the liver			
Fold increase compared to control			
1.91	**Egr1**	Early growth response 1	NM_012551
1.82	Bhlhe40	Basic helix-loop-helix family, member e40	NP_003661
1.46	Nr1d1	Nuclear receptor subfamily 1, group D, member 1	NM_145775
1.35	Creb3	CAMP responsive element binding protein 3	NM_001013092
1.34	Tcfap2a	Transcription factor AP-2, alpha	NM_001107345
Fold increase compared to control			
-1.26	Aanat	Arylalkylamine N-acetyltransferase	NM_012818
-1.27	Chrnb2	Cholinergic receptor, nicotinic, beta 2 (neuronal)	NM_019297
-1.39	Arntl2	Aryl hydrocarbon receptor nuclear translocator-like 2	NP_001234931
-1.61	Epo	Erythropoietin	NM_017001
-1.68	Prkar1b	Protein kinase, cAMP dependent regulatory, type I, beta	NM_001033679
Effect of food reward and food restriction on gene expression in the liver			
Fold increase compared to control			
46.40	Per3	Period homolog 3 (Drosophila)	NM_023978
29.05	Dbp	D site of albumin promoter (albumin d-box) binding protein	NM_012543
14.49	Nr1d1	Nuclear receptor subfamily 1, group D, member 1	NM_145775
9.86	Nr1d2	Nuclear receptor subfamily 1, group D, member 2	NM_147210
6.74	Per1	Period homolog 3 (Drosophila)	NM_001034125
Fold increase compared to control			
-2.21	Ldha	Lactate dehydrogenase A	**NM_017025**
-2.27	Stat5a	Signal transducer and activator of transcription 5A	NM_017064
-2.58	Srebf1	Sterol regulatory element binding transcription factor 1	NM_001276707
-5.19	Prkacb	Protein kinase, cAMP dependent, catalytic, beta	NM_001077645
-19.49	Arnt1	Aryl hydrocarbon receptor nuclear translocator-like	NM_024362

1st column – fold change in expression (treatment/control); 2nd column – gene abbreviation; 3rd column – gene name; 4th column – GenBank accession number.

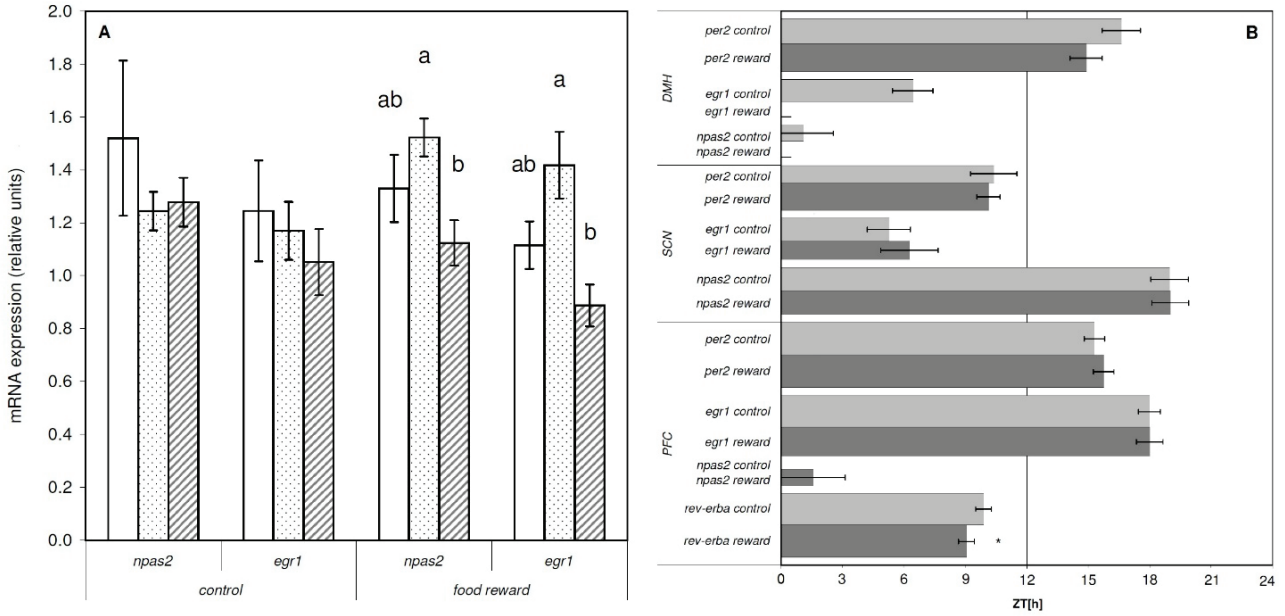

Fig. 1. Effect of food reward on (**A**) *egr1* and *npas2* expression in the DMH of rats synchronized to a 12:12 LD cycle (n=5-6). Food rewards were provided over 16 days 3 h after the beginning of the LD cycle (ZT3). White columns – ZT2; dotted columns – ZT4 (1 h after food reward delivery); cross-hatched columns – ZT6. The effect of food reward at ZT4 was compared to levels at ZT2 and ZT6. Columns with different alphabetical labels are significantly different (ANOVA). (**B**) Acrophases of *egr1*, *npas2*, *rev-erba* and *per2* expression in the suprachiasmatic (SCN), arcuate (ARC) and dorsomedial (DMH) hypothalamic nuclei and prefrontal cortex (PFC) of control (gray columns) and rewarded (dark gray columns) rats (n=5-6). Timescale is given in Zeitgeber time. The thickness of the columns corresponds to the goodness of fit (Table 2). * P<0.05 t-test comparison of acrophases.

The expression of *per2* in the SCN of the control group showed an expected pattern and food reward numerically increased goodness of fit of rhythmic *per2* expression (Fig. 1B, cosinor, Table 2). The expression of *egr1* in the SCN showed a significant rhythm with a peak in the middle of the light phase (Fig. 1B, cosinor, Table 2), and food reward caused a numerical decrease in the goodness of fit of *egr1* rhythmic expression. *npas2* exerted a significant daily rhythm in the SCN with the maximum in the middle of the dark part of the LD cycle in both groups.

The expression of *per2*, *npas2* and *egr1* did not show changes in response to food reward in the ARC (cosinor, Table 2).

The expression of *per2*, *npas2* and *egr1* in the liver of the control group showed a rhythmic pattern and food reward caused a diminishing of the rhythm in *egr1* expression. Food reward together with food restriction caused a significant change in acrophase in *per2* and *npas2* rhythmic expression in the liver (cosinor, Table 2).

The most pronounced changes in response to food reward were observed in the PFC. Food reward caused a decrease in the amplitude of the *per2* daily profile, increased the mesor of *egr1* rhythm, caused a significant change in acrophase in the *rev-erba* daily profile and induced rhythm in *npas2* expression (Fig 1B, Fig. 2, cosinor, Table 2).

Discussion

Gene expression profiling revealed that DNA-binding transcriptional factor with zinc finger *egr1* (early growth response protein 1) and bHLH-PAS transcription factor *npas2* can be involved in food reward-induced effects. Food reward provided during a passive phase of the LD cycle strongly influenced the expression of *egr1* and *npas2* predominantly in the PFC and DMH. The most interesting finding is the induction of *npas2* daily rhythm in the PFC in rewarded animals.

The provision of a food reward during the light time did not disrupt *per2* rhythmic expression in any tissue. We observed an increase in the goodness of fit of the rhythm in *per2* expression in the SCN and glycemia. Rhythm in locomotor activity showed high levels during the dark time. These findings imply that the circadian system used food reward to support the dominant synchronizing signal, which in this case is the LD cycle. As expected, food reward together with food restriction caused a huge change in acrophase in gene expression in the liver, which is not observed after treatment with food reward alone.

Table 2. Daily pattern of mRNA expression and glycemia in control and rewarded rats analyzed by cosinor analysis.

			Acrophase (h:mm)	Acrophase SEM (h:mm)		Amplitude (a.i.)	Amplitude SEM (a.i.)		Mesor (a.i.)	Mesor SEM (a.i.)		Goodness of fit	P
DMH	*per2*	control	**16:37**	00:56		**0.313**	0.066		**1.22**	0.051		**0.652**	0.000
		reward	**14:54**	00:47		**0.392**	0.070		**1.16**	0.054		**0.715**	0.000
	egr1	control	**06:26**	00:59		**0.330**	0.082		**0.90**	0.060		**0.590**	0.001
		reward	**ns**	ns		**ns**	ns		**ns**	ns		**0.393**	0.080
	npas2	control	**01:05**	01:28		**0.235**	0.090		**1.21**	0.064		**0.432**	0.041
		reward	**ns**	ns		**ns**	ns		**ns**	ns		**0.411**	0.063
SCN	*per2*	control	**10:23**	01:08		**0.248**	0.087		**0.54**	0.058		**0.461**	0.028
		reward	**10:08**	00:34		**0.269**	0.048		**0.49**	0.032		**0.709**	0.000
	egr1	control	**05:16**	01:03		**0.622**	0.153		**1.37**	0.117		**0.598**	0.001
		reward	**06:16**	01:24		**0.372**	0.129		**1.36**	0.095		**0.466**	0.023
	npas2	control	**18:58**	00:55		**1.126**	0.268		**3.70**	0.192		**0.608**	0.001
		reward	**19:00**	00:54		**0.980**	0.230		**3.56**	0.164		**0.614**	0.001
ARC	*per2*	control	**15:25**	00:54		**0.260**	0.052		**0.79**	0.041		**0.669**	0.000
		reward	**15:00**	00:56		**0.262**	0.055		**0.86**	0.043		**0.652**	0.000
	egr1	control	**ns**	ns		**ns**	ns		**ns**	ns		**0.330**	0.168
		reward	**ns**	ns		**ns**	ns		**ns**	ns		**0.327**	0.173
	npas2	control	**23:54**	1:16		**0.119**	0.043		**0.77**	0.029		**0.448**	0.031
		reward	**00:40**	1:24		**0.145**	0.055		**0.82**	0.039		**0.434**	0.039
PFC	*per2*	control	**15:18**	00:30		**0.064**	0.007		**0.13**	0.006		**0.850**	0.000
		reward	**15:45**	00:30		**0.050**	0.006	*	**0.13**	0.004		**0.852**	0.000
	egr1	control	**17:59**	00:32		**0.289**	0.037		**0.60**	0.028		**0.822**	0.000
		reward	**18:00**	00:39		**0.292**	0.047		**0.68**	0.035	*	**0.751**	0.000
	npas2	control	**ns**	ns		**ns**	ns		**ns**	ns		**0.140**	0.737
		reward	**01:34**	01:34		**0.102**	0.040		**0.57**	0.029		**0.421**	0.048
	rev-erba	control	**09:53**	00:23		**0.638**	0.076		**1.22**	0.050		**0.833**	0.000
		reward	**09:03**	00:23	*	**0.713**	0.084		**1.27**	0.056		**0.836**	0.000
Liver	*per2*	control	**16:44**	00:21		**0.463**	0.038		**0.60**	0.029		**0.911**	0.000
		reward	**16:31**	00:16		**0.478**	0.029		**0.60**	0.023		**0.947**	0.000
	egr1	control	**10:34**	01:19		**0.161**	0.065		**0.37**	0.043		**0.412**	0.061
		reward	**ns**	ns		**ns**	ns		**ns**	ns		**0.129**	0.770
	npas2	control	**03:51**	00:22		**0.527**	0.043		**0.41**	0.033		**0.911**	0.000
		reward	**03:54**	00:23		**0.563**	0.050		**0.41**	0.039		**0.898**	0.000
	per2	control	**16:50**	00:34		**2.068**	0.302		**2.32**	0.214		**0.870**	0.000
		restriction	**05:00**	00:46	*	**1.438**	0.288	*	**1.86**	0.204	*	**0.736**	0.000
	npas2	control	**01:10**	00:36		**0.249**	0.039		**0.20**	0.027		**0.857**	0.000
		restriction	**14:28**	00:48	*	**0.313**	0.066		**0.21**	0.047		**0.719**	0.001
Plasma	*glucose*	control	**ns**	ns		**ns**	ns		**ns**	ns		**0.347**	0.137
		reward	**17:47**	01:26		**1.566**	0.532		**10.62**	0.402		**0.470**	0.021

Mesor – average value of fitted curve; Amplitude – value of curve peak relative to mesor; Acrophase – time of curve peak from circadian time zero (dark-to-light transition) in hour:minutes. Mesor and amplitude are given in relative units (a.i.). Amplitude is given in hour:minutes (h:mm). ns – nonsignificant daily change. SCN – suprachiasmatic, ARC – arcuate, DMH – dorsomedial nuclei, PFC – prefrontal cortex. * $P<0.05$, cosinor.

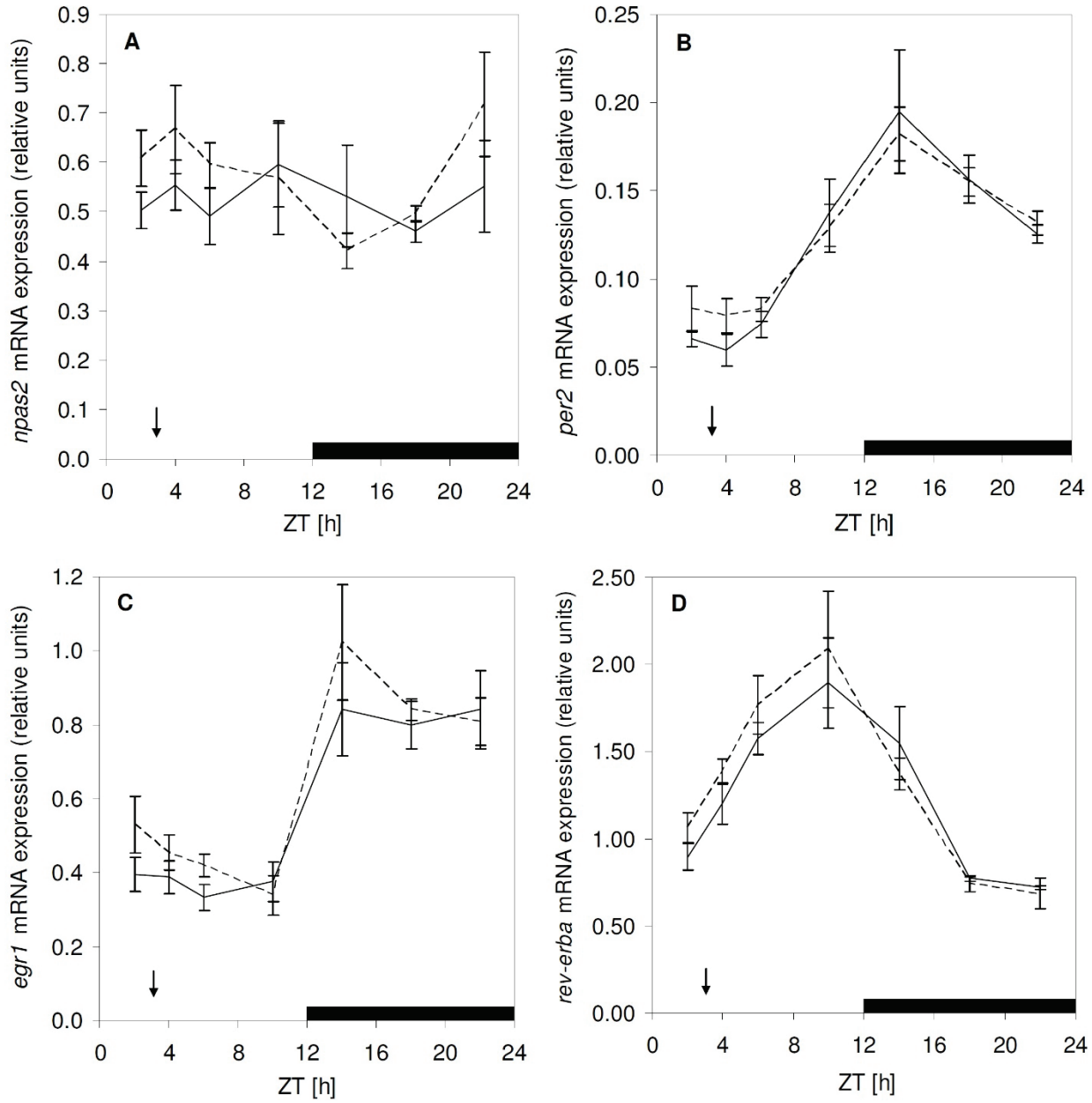

Fig. 2. Daily profile of *npas2* (**A**), *per2* (**B**), *egr1* (**C**) and *rev-erba* (**D**) in the prefrontal cortex of control rats (solid line) and rats provided with food rewards over 16 days at ZT3 (broken line). The rats were exposed to a 12:12 LD cycle. The black arrows indicate food reward delivery. The black bars at the bottom of the charts indicate the dark part of the LD cycle.

The effect of food reward on clock gene expression during a 24 h cycle was studied predominantly in the limbic system and brain regions involved in energy homeostatic regulation (Verwey *et al.* 2007, Verwey *et al.* 2008, Mendoza *et al.* 2010, Challet and Mendoza 2010). In our study, the expression of *per2* showed a distinct daily rhythm as in the DMH of rats (Monošíková *et al.* 2007, Minana-Solis *et al.* 2009) and mice (Moriya *et al.* 2009) with the same acrophase as it was reported earlier. It is well known that food restriction strongly amplifies *per* expression and the amplitude of its rhythm in the DMH (Verwey *et al.* 2007, Moriya *et al.* 2009, Verwey *et al.* 2008). Compared to this effect, the change in *per2* expression after food reward is much less pronounced in this structure.

There was a distinct daily pattern in *egr1* and *npas2* expression in the DMH in the control group. Food reward caused an increase in *egr1* and *npas2* expression 1h after reward delivery that led to rhythm diminishing. This finding implies that the DMH, which is involved in the regulation of wakefulness and responsiveness to environmental cues that are important to the animal, can facilitate food reward acceptance at the right time.

Food reward did not induce change either in the

per2 daily pattern as reported earlier (Moriya *et al.* 2009) or in *egr1* and *npas2* expression in the ARC.

We observed a pronounced daily rhythm in *per2*, *npas2* and *egr1* expression in the liver with the maximum as was reported before (Damiola *et al.* 2000, Tao *et al.* 2015). *per2* and *npas2* expression was not influenced by food reward; however, food reward caused a diminishing of *egr1* rhythmic expression. Food reward with food restriction caused a pronounced phase shift in gene expression as was reported earlier (Damiola *et al.* 2000, Tao *et al.* 2015).

The PFC, which receives input from the mesolimbic system, is involved in executive behavioral response and influences behavioral strategy preservation (Reick *et al.* 2001, Dudley *et al.* 2003). The induction of *npas2* rhythm is most probably related to one of these PFC functions, allowing animals to accept food reward during a passive phase without major disturbance of the rhythm in locomotor activity. The rhythmic pattern of *egr1* in the PFC showed increased mesor in rewarded animals. The expression of *rev-erba* showed a significant change in acrophase and trend to increased amplitude. *rev-erba* has lately proved to be crucial for food restriction-induced behavior. Since *npas2* contains the binding domain for *rev-erba* it is possible that change in *rev-erba* expression contributed to the changed pattern in *npas2* expression (Delezie *et al.* 2016).

Unlike the DMH, changes in *egr1* and *npas2* expression in the PFC do not show an acute response at the time of food reward delivery. Changes observed in gene expression influenced the daily pattern.

While *npas2* is known for its role in the circadian oscillator function, *egr1* is known mainly as an immediate early gene involved in neural development, cell differentiation, memory and learning (Beckmann and Wilce 1997). Its direct connection to the circadian system was recently established when functional E-box was identified in its sequence (Tao *et al.* 2015). In spite of the presence of functional E-box, *egr1* shows surprising variability in its acrophase in tissues with similar expression of the BMAL1 (NPAS2)/CLOCK heterodimer. This variability implies more complex e*gr1* regulation, which is supported by the presence of a variety of response elements in the *egr1* promoter (Meyer *et al.* 2002).

The PFC is known to play a role in reward response (Warren *et al.* 2016) after activation of the mesolimbic system (Webb *et al.* 2015). Changes in gene expression in the DMH could be induced by input from the lateral hypothalamus, which is in addition to the regulation of food behavior also involved in the regulation of sleep and wakefulness, food reward responsiveness, addiction and energy homeostasis (Feillet *et al.* 2015). These functions are regulated *via* afferentation from the limbic system and by humoral satiety signals, metabolic cues and glucose from the periphery (Sakurai *et al.* 2007). In both cases a possible link between changed genes expression and exact mechanism of molecular signaling leading to changed behavior needs to be elucidated.

To conclude, food reward of low hedonic and caloric value provided during a passive phase of the LD cycle induced *npas2* rhythm in the PFC and increased *npas2* and *egr1* expression in the DMH. The DMH can influence the SCN to modulate the pattern of wakefulness and the PFC most probably contributes to executive behavior during reward presentation. We observed an improved rhythmicity in *per2* expression in the SCN and plasma glucose levels. These findings imply that regularly administered food rewards of small hedonic and caloric value can reinforce entrainment of the circadian system to the actual LD cycle even if the reward is presented in a passive phase of the LD cycle.

Conflict of Interest

There is no conflict of interest.

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