ORIGINAL ARTICLE

Tissue specific regulation of *egr1* rhythmic expression in the prefrontal cortex in comparison with other rat brain regions

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Abstract

OBJECTIVES: The circadian system regulates expression of wide range of transcriptional factors. Among them egr1 was shown to be under circadian control executed via E-box. However, tissue specificity of E-box mediated control was not investigated until now.
DESIGN: We focused on the efficiency of circadian regulation of *egr1* at both sides of blood-brain barrier, particularly in the brain and in peripheral organ.
SETTING: Expression of *egr1* was investigated during 24h LD cycle in four brain regions including suprachiasmatic nucleus (SCN), dorsomedial hypothalamus (DMH), arcuate nucleus (ARC) and prefrontal cortex (PFC). Expression in the brain was compared to expression in the heart and liver. Characteristics of daily rhythm of *egr1* expression were correlated to expression of core clock gene *per2*.
RESULTS: Expression of *egr1* showed a rhythmic pattern in 5 out of 6 investigated tissues. Peak of *egr1* rhythmic expression in the PFC was phase delayed compared to peak of *per2* rhythm in this tissue. On the other hand, rhythm in *egr1* expression was phase advanced in the SCN, DMH, liver and the heart in comparison to *per2* expression. Expression of *egr1* was arrhythmic in the ARC in spite of rhythmic *per2* expression.
MAIN FINDINGS: Our data clearly demonstrate that even with functional E-box in gene promoter, final acrophase of *egr1* expression can show strong tissue dependent changes in rhythm parameters within the brain.
CONCLUSIONS: We suppose that other regulation regions than E-box can contribute to final tissue specific pattern of *egr1* rhythmic expression.

Abbreviations:
AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP1 - activator protein 1; ARC - arcuate nucleus; CRE – cAMP regulatory element; *egr1* - early growth response protein 1; DMH - dorsomedial nucleus; NAB1, NAB2 - NGFI-A binding proteins 1 and 2; NMDA - N-methyl-D-aspartate; SCN - suprachiasmatic nucleus; PFC - prefrontal cortex; SP1 - transcription factor Sp1; SRE - serum response element

Introduction

Early growth response 1 transcriptional factor (Egr1 known also as NGFI-A, Zif/268, Tis8 or Krox24) belongs to the immediate early gene family (Beckmann & Wilce 1997). The typical feature of *egr* family is a highly conserved DNA-binding domain that is structured of three zinc-finger motifs. EGR via its DNA binding domain recognizes a nine-base-pair CG rich segment of DNA – EGR response element (Donovan *et al* 1999). EGR1 is promptly and transiently induced in response to growth factors, cytokines, hypoxia, cell stress and other factors (Meyer *et al* 2002) and influences activity of dozens genes (Svaren *et al* 2000; Arora *et al* 2008). Regulation of *egr1* transcription is mediated via several known promoter sequences, including several SREs, at least one CRE (cAMP regulatory element), several SP1 consensus sequences, AP1-like element, hepatocyte nuclear factor 4 (HNF4) binding sites (Herdegen & Leah 1998; Zhang *et al* 2011; Meyer *et al* 2002) and some others (Veyrac *et al* 2014). Regulation of *egr1* transcription also involves a negative transcriptional feedback loop as the protein product of *egr1* represses its own transcription through binding to the EGR response element (Cao *et al* 1993). The biological activity of *egr1* is blocked by two co-factors NGFI-A binding proteins 1 and 2 (NAB1, NAB2) that bind to the inhibitory domain within the Egr1 (Veyrac *et al* 2014). Lately, E-box was identified in *egr1* promoter (Tao *et al* 2015). This finding implicates direct connection between *egr1* expression and the circadian system.

The circadian system regulates endogenous rhythms in physiology and behavior with 24h period. Functional organization of the circadian system involves central (master) and peripheral oscillators. In mammals, the master oscillator is localized in the suprachiasmatic nucleus (SCN) located in the anterior hypothalamus and synchronizes autonomous peripheral oscillators distributed in the body. It is assumed that molecular basis of the circadian rhythms generation is transcription-translation negative feedback loop. The core of the loop is created by *period (per1, per2, per3*) and *cryptochrome (cry1, cry2*) genes (so-called clock genes) that can inhibit their own transcription. *per* and *cry* expression is induced by heterodimer of two basic helix-loop-helix transcription factors CLOCK and BMAL1 via regulatory element E-box (Albrecht 2012). Protein products of *per* and *cry* genes accumulate in the cytoplasm and translocate to the nucleus, where they repress their own transcription by interacting with heterodimer CLOCK-BMAL1. To complete this feedback loop takes approximately 24h (Reppert & Weaver 2002). Expression of the majority of core clock genes exhibits robust circadian rhythms in the SCN and in the most of the peripheral tissues with the well preserved phase shift between master and peripheral oscillators (Yamamoto *et al* 2004; Ko &Takahashi 2006).

Transcriptional factors involved in the basic loop regulate via E-box hundreds of clock-controlled genes (ccg) showing a tissue specific rhythmic pattern in expression (Panda *et al* 2002). Tissue specificity of rhythmically expressed ccg is very likely associated with their function in the particular tissue (Storch *et al* 2002).

egr1 is ubiquitously expressed in mammalian tissues (Yu *et al* 2014; Ardlie *et al* 2015). In the brain *egr1* transcription is the most frequently investigated in context of neural development, cell differentiation, passive and active learning and memory and somatosensory stimulation (Beckmann & Wilce 1997; Herdegen & Leah 1998). *egr1* as well as other immediate early genes can be induced by photic stimuli (Rusak *et al* 1990; Ebling *et al* 1991). EGR1 mediated regulatory pathway has been also implicated in lithium dependent activation of *per2* transcription through binding to its promoter. This is supported also by the fact, that induction of *per2* by lithium was attenuated in *egr1*–/– mice (Kim *et al* 2013). *egr1* activates also *per1* transcription in mouse hepatocytes by binding to the proximal region of *per1* promoter and modulates the expression of other clock genes, including *bma1*, *per2* or *Reverbα* (Tao *et al* 2015).

The aim of our study is to elucidate how tightly E-box that competes with influences of many other regulatory regions in the *egr1* promoter regulates its expression at both sides of blood-brain barrier, particularly in the brain and in peripheral organs. This approach has been selected since internal milieu in the brain and other tissues differs strongly in respect to *egr1* regulatory factors.

Material & Methods

Male Wistar rats (n=36) were obtained from VELAZ Praha (Czech Republic) at the age of 10–11 weeks. Animals were housed in a temperature controlled rooms (21±2 °C) under the LD cycle 12:12 in cages occupied by 4 animals to allow social interactions. Food and water were available *ad libitum*. Samples of liver, heart, blood and whole brains were taken during whole 24 h cycle after 4 weeks of this treatment. Sampling during the dark phase of LD cycle occurred in dark red light with low intensity. Prefrontal cortex (PFC), liver and heart tissues were immediately after dissection frozen in liquid nitrogen and stored under –80 °C until total RNA isolation. Brains were frozen on dry ice and tissues were stored under –80 °C until further processing. Selected brain areas (SCN, dorsomedial nucleus – DMH and arcuate nucleus – ARC) were dissected bilaterally by the micropunch technique according to the punching guide atlas (Palkovits & Brownstein 1998) as described previously (Monosikova *et al* 2007, Herichova *et al* 2013). Briefly, brains were cut into 300 μm thick serial coronal sections in a cryostat (Reichert-Jung, Leica, Germany) at –10 °C. The SCN, ARC and DMH were isolated under the dissection microscope by special metal punching needles with diameter of

400 µm and punches from individual rats were stored at –80 °C until the RNA isolation.

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 European Communities Council Directive of 24 November 1986 (86/609/EEC).

Total RNA from tissues was isolated using Tri reagentR (MRC, USA). RNA from the brain punches was isolated using 0.5 µl coprecipitant GlycoBlue (Ambion, USA) according to the manufacturer's instructions. The first-strand cDNA synthesis was carried out with the use of the ImProm-IITM Reverse Transcription System (Promega, USA). The quantification of cDNA was performed by real time PCR using the QuantiTect SYBR Green PCR kit (Qiagen, Germany) and Step-One System (Applied Biosystems, USA). The primer pairs used for the amplification of *per2* and *rplp1* and real time PCR conditions were as described previously (Herichova *et al* 2013). The other primer pairs used for the amplification were: *egr1* (Databaze ID: NM_012551.2) sense 5'-AACAACCCTACGAGCACCTG-3', antisense 5'-GGGTAGTTTGGCTGGGATAA-3'. Real time PCR conditions for *egr1* measurement were: hot start 95 °C for 15 min followed by 40 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s. The specificity and identity of the PCR products were validated by melting curve analysis. Gene expression was normalized to *rplp1* expression. The fluorescence dye ROX served as an internal reference for normalization of SYBR Green I fluorescent signal.

Statistics

Statistical analysis was performed by cosinor analysis. Data were fitted into a cosinor curve with 24h period and when experimental data significantly matched the cosinor curve, its parameters were calculated with 95% confidence limits: mesor (the time series mean), amplitude (one half of the peak-trough difference expressed herein relative to the mesor), and acrophase (peak time referenced to the time of lights on in the animal facility). Time is expressed in relative units – Zeitgeber time (ZT), when ZT0 is defined as beginning of the light phase of day.

Results

The best calculated fits (cosinor) of *per2* and *egr1* expression in the brain tissues (A, C) and peripheral tissues (B, D) are shown at Figure 1. Daily rhythm in *per2* expression was significant in the central oscillator and all other brain tissues as well as in peripheral tissues (cosinor, $p<0.05$). Acrophase of *per2* expression in the SCN peaked at the end of the light part of LD cycle while peak of *per2* expression in the DMH, ARC, PFC, heart and liver was observed at the beginning of the dark part of the LD cycle.

Acrophases of rhythmic *egr1* expression showed much wider range than it was observed in *per2* rhythmic expression. We observed a significant daily rhythm in *egr1* expression in the SCN, DMH, PFC, liver and the heart (cosinor, $p<0.05$), however, *egr1* expression was not rhythmic in the ARC (cosinor). Expression of *egr1* in the SCN, DMH and the heart peaked approximately in the middle of the L phase of the LD cycle. Maximum of *egr1* expression was observed at the transition from L to D phase of LD cycle in the liver. Only PFC exerted maximum of *egr1* rhythmic expression in the dark phase of LD cycle.

Differences in phase angles between *per2* and *egr1* rhythmic expression (Figure 2) corresponds well with differences in *egr1* acrophases. Calculation of phase angle between *per2* and *egr1* expression in the PFC indicates a delayed peak of *egr1* expression in comparison with *per2* rhythm. Rhythmic expression of *egr1* was phase advanced in relation to *per2* rhythmic expression in the SCN, liver, DMH and the heart.

Discussion

It is generally accepted that prominent part of regulatory influence of the circadian system on transcriptome is mediated via regulatory region called E-box (Albrecht 2012). It is also well known that robust rhythmic clock controlled gene expression shows a strong tissue specificity (Panda *et al* 2002). Here we report that acrophase of rhythmic expression of particular gene also exerts tissue specificity.

Expression of the transcriptional factor *egr1* and clock gene *per2* was investigated in the SCN, DMH, ARC, PFC, heart and liver of rats synchronized to LD cycle 12:12. Expression of core clock gene *per2* showed an expected rhythmic pattern in all investigated tissues (Yamamoto *et al* 2004, Monosikova *et al* 2007, Herichova *et al* 2013) with well preserved phase angle between master and peripheral oscillators. Unlikely *per2*, *egr1* showed wide range of acrophases with tissue specific phase angles between rhythmic *per2* and *egr1* expression.

Daily rhythm in *egr1* expression was previously described in the SCN (Guido *et al* 1999) and liver (Tao *et al* 2015) of rat. *egr1* expression was higher during the day than during the night in the SCN (Guido *et al* 1999) and peak of rhythmic *egr1* expression was observed in the second half of the light phase of 24h cycle in the liver (Tao *et al* 2015). These findings are in accordance with our recent observations. Acrophase of daily pattern in *egr1* expression was not described in the ARC, DMH a heart according to our knowledge till now.

Egr1 is transcriptional factor with ubiquitous basal expression in mammals (Yu *et al* 2014; Ardlie *et al* 2015). Basal *egr1* expression is regulated via several regulatory domains (Knapska & Kaczmarek 2004) including E-box (Tao *et al* 2015). Functional E-box in *egr1* promoter was proved in the liver of mice (Tao *et*

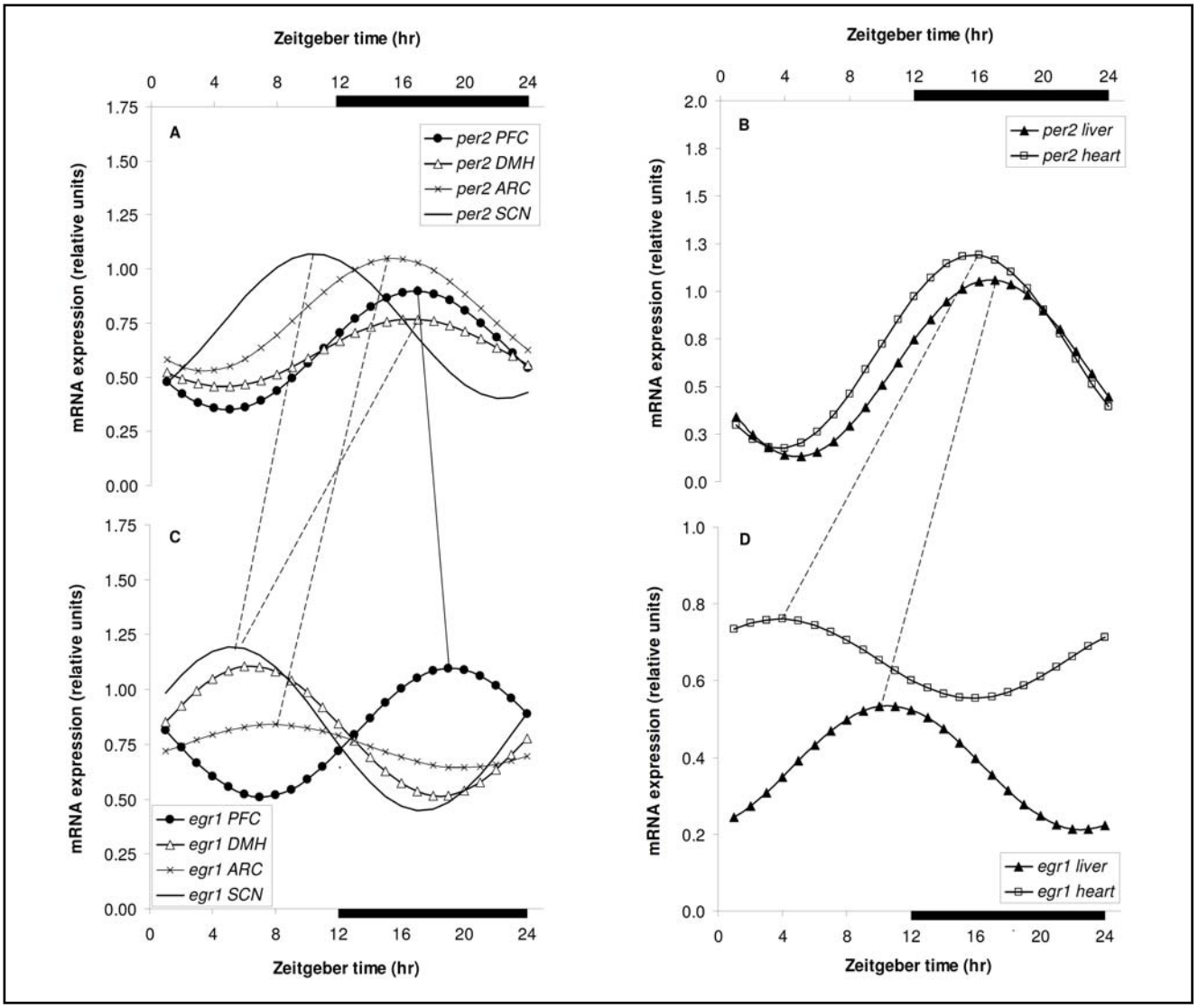
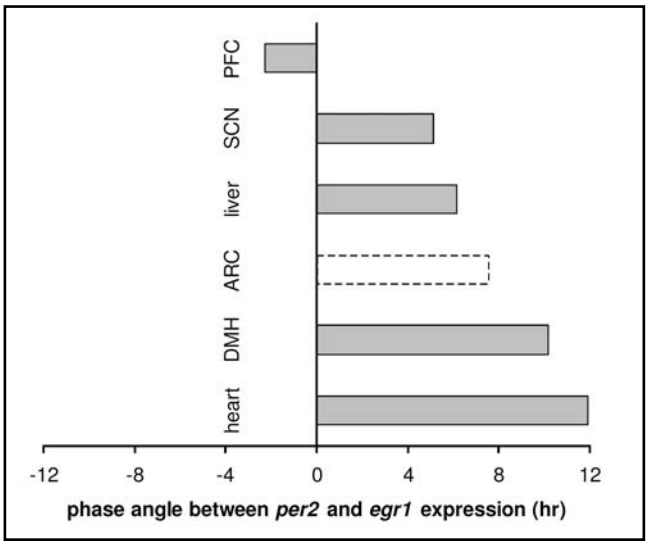


Fig. 1. The best calculated fits of *per2* (A, B) and *egr1* (C, D) expression in the SCN (suprachiasmatic nuclei), DMH (dorsomedial hypothalamic nuclei), ARC (nucleus arcuatus), PFC (prefrontal cortex), heart and liver of rats synchronized to LD cycle 12:12. Dark bars at the top and bottom of the graphs express dark part of 12:12 LD cycle. Broken and full lines connecting chards A–C and B–D, respectively, indicates phase angle between *per2* and *egr1* 24h expression. Type of line (broken *vs.* full) implicates difference in orientation of phase angle. Time is expressed in relative time units – Zeitgeber time, where ZT0 correspond to the beginning of the L phase.



al 2015) that implicates similar mechanism of circadian regulation of *egr1* transcription in other tissues as well. However, our findings do not support this hypothesis. We suppose that influence of E-box is modified by other factors to general final pattern of tissue specific rhythmic *egr1* expression.

egr1 rhythm in expression showed a delayed peak in the PFC and advanced peak in the SCN, liver, DMH and the heart in relation to maximum of *per2* expres-

Fig. 2. Phase angle between *per2* and *egr1* expression in the SCN (suprachiasmatic nuclei), DMH (dorsomedial hypothalamic nuclei), ARC (nucleus arcuatus), PFC (prefrontal cortex), heart and liver of rats synchronized to LD cycle 12:12. Gray columns shows phase angle calculated from two significant cosinor curves, while white column bordered by broken line indicates hypothetical phase angle in the ARC where expression of *per2* was significant but expression of *egr1* was not.

sion. Presence of blood-brain barrier does not seem to be a critical factor for determination of phase angle between *per2* and *egr1* expression on the basis of our results since SCN, DMH and liver showed similar phase angles between *per2* and *egr1* daily rhythms.

A reason why *egr1* expression shows a delay in relation to maximum of *per2* expression only in the PFC is unclear. We can only hypothesize that unique acrophase phase of rhythmic expression of *egr1* in PFC is related to role of this brain structure in cognitive and executive processes that are extensively studied in mammals including human (Ferguson & Gao 2015). Prefrontal cortex firing was experimentally associated with establishment of balance between fear extinction and preservation (Fitzgerald *et al* 2014). PFC in rodents has been shown to be related to optimal scheduling of complex sequences of behavior related to executive functioning that includes attentional selection, resistance to interference, monitoring, behavioral inhibition, task switching, planning and decision making (Dalley *et al* 2004). All these processes are prone to be activated much more frequently during the active part of 24h cycle that is a dark period in rat.

egr1 expression in the brain is induced by NMDA and AMPA receptors and L-type voltage-sensitive calcium channels that implicates that expression of *egr1* is triggered by synaptic activity induced by some stimulus (Veyrac *et al* 2014). This is generally confirmed by many experimental studies demonstrating induction of *egr1* expression in response to wide range of physiological and stress stimuli (Duclot & Kabbaj 2017). In this respect, *egr1* expression in the brain has been linked to maintenance of long term potentiation (LTP) (Jones *et al* 2001), memory consolidation and learning (Knapska & Kaczmarek 2004).

egr1 expression in PFC was investigated predominantly with contextual fear conditioning accompanied by increase in *egr1* expression (Schreiber *et al* 2014). In animals exposed to foot-shock a preexposure facilitation effect paradigm proved that *egr1* expression in the prefrontal cortex correlates with development of contextual fear (Asok *et al* 2013). It was shown that medial PFC is involved in recent and remote memory recall in fear conditioning in rats and that is accompanied by induction in *egr1* expression (Blum *et al* 2006). Depression of synaptic transmission and failure of induction *egr1* in mPFC is associated with improved recovery of conditioned fear in mice (Herry & Mons 2004). *egr1* expression is involved in the formation of episodic-like memory in context to object recognition with or without same position in the open field (Barbosa *et al* 2013). Underlying the role of PFC and *egr1* in learning, it was shown that *egr1* expression increases in medial PFC in animals exposed to novel objects (Yochiy *et al* 2012).

To conclude, recent knowledge is not sufficient to explain how specific acrophases of *egr1* expression are generated in the body with one master oscillator. This phenomenon implicitly requires cumulative effect of several regulatory factors. *egr1* has been previously shown to regulate gene expression in a tissue specific manner (Duclot & Kabbaj 2017) and we suppose that existence of rhythm in *egr1* expression with tissue specific acrophases can, to some extend, support this observation. Surprisingly, acrophase of *egr1* rhythmic expression in the PFC differs strongly compared to other studied brain tissues and peripheral tissues. We speculate that high expression of *egr1* in the PFC during the dark period can reflect endogenous capacity of PFC to achieve threshold value in *egr1* expression to induce cell response to stimulus that is more likely to occur during the dark part of LD cycle in rat. However, this idea needs to be further investigated.

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REFERENCES

1 Albrecht U (2012) Timing to perfection: the biology of central and peripheral circadian clocks. *Neuron.* **74**: 246–260.
2 Arora S, Wang Y, Jia Z, Vardar-Sengul S, Munawar A, Doctor KS, Birrer M, McClelland M, Adamson E, Mercola D (2008). Egr1 regulates the coordinated expression of numerous EGF receptor target genes as identified by ChIP-on-chip. *Genome Biol.* **9**: R166.
3 Asok A, Schreiber WB, Jablonski SA, Rosen JB, Stanton ME (2013). Egr-1 increases in the prefrontal cortex following training in the context preexposure facilitation effect (CPFE) paradigm. *Neurobiol Learn Mem.* **106**: 145–153.
4 Barbosa FF, Santos JR, Meurer YS, Macêdo PT, Ferreira LM, Pontes IM, Ribeiro AM, Silva RH (2013) Differential Cortical c-Fos and Zif-268 Expression after Object and Spatial Memory Processing in a Standard or Episodic-Like Object Recognition Task. *Front Behav Neurosci.* **7**: 112.
5 Beckmann AM, Wilce PA (1997). Egr transcription factors in the nervous system. *Neurochem Int.* **31**: 477–510.
6 Blum S, Hebert AE, Dash PK (2006) A role for the prefrontal cortex in recall of recent and remote memories. *Neuroreport.* **17**: 341–4.
7 Cao X, Mahendran R, Guy GR, Tan YH (1993). Detection and characterization of cellular EGR-1 binding to its recognition site. *J Biol Chem.* **268**: 16949–16957.
8 Dalley JW, Cardinal RN, Robbins TW (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev.* **28**: 771–784.
9 O'Donovan KJ, Tourtellotte WG, Millbrandt J, Baraban JM (1999). The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci.* **22**: 167–173.
10 Duclot F, Kabbaj M (2017) The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. *Front Behav Neurosci.* **11**: 35.
11 Ebling FJP, Maywood ES, Staley K, Humby T, Hancock DC, Waters CM, Evan GI, Hastings MH (1991). The role of N-methyl-D-aspartate-type glutamatergic neurotransmission in the photic induction of immediate-early gene expression in the suprachiasmatic nuclei of the Syrian hamster. *J Neuroendocrinol.* **3**: 641–652.
12 Ferguson BR, Gao WJ (2015). Development of thalamocortical connections between the mediodorsal thalamus and the prefrontal cortex and its implication in cognition. *Front Hum Neurosci* **8**: 1027.
13 Fitzgerald PJ, Whittle N, Flynn SM, Graybeal C, Pinard CR, Gunduz-Cinar O, Kravitz AV, Singewald N, Holmes A (2014). Prefrontal single-unit firing associated with deficient extinction in mice. *Neurobiol Learn Mem.* **113**: 69–81.

14 GTEx Consortium, Ardlie KG, Deluca DS, Segrè AV, Sullivan TJ, Young TR, *et al* (2015). Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science.* **348**: 648–660.
15 Guido ME, de Guido LB, Goguen D, Robertson HA, Rusak B (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *J Biol Rhythms.* **14**: 275–280.
16 Herdegen T, Leah JD (1998). Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Res Brain Res Rev.* **28**: 370–490.
17 Herichova I, Soltesova D, Szantoova K, Mravec B, Neupauerová D, Vesela A, Zeman M (2013). Effect of angiotensin II on rhythmic per2 expression in the suprachiasmatic nucleus and heart and daily rhythm of activity in Wistar rats. *Regul Pept.* **186**: 49–56.
18 Herry C, Mons N. (2004). Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. *Eur J Neurosci.* **20**: 781–790.
19 Jones MW, Errington ML, French PJ, Fine A, Bliss TV, Garel S, Charnay P, Bozon B, Laroche S, Davis S (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nat Neurosci.* **4**: 289–296.
20 Kim SH, Yu HS, Park HG, Ahn YM, Kim YS, Lee YH, Ha K, Shin SY (2013). Egr1 regulates lithium-induced transcription of the Period 2 (PER2) gene. *Biochim Biophys Acta.* **1832**: 1969–1979.
21 Knapska E, Kaczmarek L (2004). A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *Prog Neurobiol.* **74** : 183–211.
22 Ko CH, Takahashi JS (2006). Molecular components of the mammalian circadian clock. *Hum Mol Genet.* Spec No **2**: R271–277.
23 Meyer RG, Kupper JH, Kandolf R & Rodemann HP (2002) Early growth response-1 gene (Egr-1) promoter induction by ionizing radiation in U87 malignant glioma cells in vitro. *Eur J Biochem*. **269**: 337–346.
24 Monosikova J, Herichova I, Mravec B, Kiss A, Zeman M (2007) Effect of upregulated renin-angiotensin system on per2 and bmal1 gene expression in brain structures involved in blood pressure control in TGR(mREN-2)27 rats. *Brain Res.* **1180**: 29–38.
25 Palkovits M & Brownstein MJ (1998). Maps and Guide to Microdissection of the Rat Brain, New York: Elsevier Science Publishing Co., ISBN 0444012567, p. 1–162.
26 Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB (2002). Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell.* **109**: 307–320.
27 Rusak B, Robertson HA, Wisden W, Hunt SP (1990). Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus. *Science.* **248**: 1237–1240.
28 Reppert SM, Weaver DR (2002). Coordination of circadian timing in mammals. *Nature.* **418**: 935–941.
29 Schreiber WB, Asok A, Jablonski SA, Rosen JB, Stanton ME (2014) Egr-1 mRNA expression patterns in the prefrontal cortex, hippocampus, and amygdala during variants of contextual fear conditioning in adolescent rats. *Brain Res.* **1576**: 63–72.
30 Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature.* **417**: 78–83.
31 Svaren J, Ehrig T, Abdulkadir SA, Ehrengruber MU, Watson MA,Milbrandt J (2000). EGR1 target genes in prostate carcinoma cells identified by microarray analysis. *J Biol Chem.* **275**: 38524–38531.
32 Tao W, Wu J, Zhang Q, Lai SS, Jiang S, Jiang C, Xu Y, Xue B, Du J, Li CJ (2015). EGR1 regulates hepatic clock gene amplitude by activating Per1 transcription. *Sci Rep.* **5**: 15212.
33 Veyrac A, Besnard A, Caboche J, Davis S, Laroche S (2014). The transcription factor Zif268/Egr1, brain plasticity, and memory. *Prog Mol Biol Transl Sci.* **122**: 89–129.
34 Yamamoto T, Nakahata Y, Soma H, Akashi M, Mamine T, Takumi T (2004). Transcriptional oscillation of canonical clock genes in mouse peripheral tissues. *BMC Mol Biol.* **9**: 5–18.
35 Yochiy A, Britto LR, Hunziker MH (2012) Novelty, but not operant aversive learning, enhances Fos and Egr-1 expression in the medial prefrontal cortex and hippocampal areas of rats. *Behav Neurosci.* **126**: 826–834.
36 Yu Y, Fuscoe JC, Zhao C, Guo C, Jia M, Qing T, Bannon DI, Lancashire L, Bao W, Du T, Luo H, Su Z, Jones WD, Moland CL, Branham WS, Qian F, Ning B, Li Y, Hong H, Guo L, Mei N, Shi T, Wang KY, Wolfinger RD, Nikolsky Y, Walker SJ, Duerksen-Hughes P, Mason CE, Tong W, Thierry-Mieg J, Thierry-Mieg D, Shi L, Wang C (2014). A rat RNA-Seq transcriptomic BodyMap across 11 organs and 4 developmental stages. *Nat Commun.* **5**: 3230.
37 Zhang Y, Bonzo JA, Gonzalez FJ & Wang L (2011) Diurnal regulation of the early growth response 1 (Egr-1) protein expression by hepatocyte nuclear factor 4alpha (HNF4alpha) and small heterodimer partner (SHP) cross-talk in liver fibrosis. *J Biol Chem.* **286**: 29635–29643.