Relationship between Circadian Clock Genes and the Neurotrophic Factor Genes in Rat Hippocampus

Ratchadaporn Pramong PhD*, Piyarat Govitrapong PhD**,***, Pansiri Phansuwan-Pujito PhD*

** Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
*** Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Nakornpathom, Thailand*
**** Chulabhorn Royal Academy, Chulabhorn Graduate Institute, Bangkok, Thailand*

Background: *The hippocampus, a key brain area for learning and memory, can express clock genes with circadian fashion. The neurotrophic factors are important for cognitive function in the hippocampus. The expression of circadian clock gene and neurotrophic factor in the hippocampus would be necessary to achieve and optimize daily memory performance.*
Objective: *It is of interest to examine the daily expression patterns of neurotrophic factor genes and the link between these genes and clock genes in rat hippocampus.*
Material and Method: *Daily expression profiles of four clock genes (Per1, Cry1, Bmal1, and Rev-erb alpha) and four neurotrophic factor genes (BDNF, NGF, NT-3, and VEGF) were analyzed in the rat hippocampus at 6 hours apart by real-time PCR.*
Results: *The mRNAs of BDNF, NGF, and VEGF, but not NT-3 in hippocampus were expressed in circadian manner as well as those of clock genes. Correlation analysis revealed strong relationships between the expression of VEGF and Per1, VEGF and Rev-erb alpha, BDNF and Bmal1, and BDNF and Cry1.*
Conclusion: *The present study suggests that VEGF and BDNF are clock-controlled genes relating to specific clock genes. Therefore, further investigation on the molecular mechanisms linking cognitive processes and circadian clock in hippocampus could be necessary to clarify their functions.*

Keywords: *Hippocampus, Clock genes, Neurotrophic factors, BDNF, NGF, NT-3, VEGF, Circadian*

J Med Assoc Thai 2017; 100 (Suppl. 8): S32-S39
Full text. e-Journal: *http://www.jmatonline.com*

Circadian rhythm is an internal biological clock of organisms ranging from bacteria to mammals. The mammalian circadian rhythm is generated by the expression of clock genes and proteins in a central pacemaker, suprachiasmatic nucleus (SCN), to mediate rhythmic biological processes by regulating clock-controlled genes. Circadian locomotor output cycles protein kaput (CLOCK) and brain and muscle ARNT-like protein-1 (BMAL1) are transcriptional activators that regulate the expressions of Period (Per) and Cryptochrome (Cry) gene by binding to an E-box element present in the promoter region. PER and CRY proteins block their own transcription by inhibiting the transcriptional activity of the CLOCK: BMAL1 heterodimer and their turnover allows this cycle to restart. The circadian rhythm is stabilized by auxiliary loops related to retinoic acid receptor-related orphan receptor (ROR) alpha and REV-ERB alpha proteins, which can activate or suppress transcription of the Bmal1, respectively[1]. At the same time, the CLOCK:BMAL1 heterodimer also regulates the transcription of clock-controlled genes that contain an E-box in their promoter[2]. The expression of clock genes is not limited in the SCN but also present in the peripheral tissues and other brain regions such as hippocampus[3].

Furthermore, some molecular factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and vascular endothelial growth factor (VEGF) as well as their receptors are localized in the hippocampus. These factors are important for memory processing and also associated with circadian rhythm[4,5]. Several studies have already demonstrated that BDNF is implicated in the regulation of circadian pacemaker function in the SCN[6-8]. In addition, injection of NGF to SCN caused phase shifts of the free-running circadian rhythm[9]. More recently, disruption of circadian clock induced a

Correspondence to:
Phansuwan-Pujito P, Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand.
Phone: +66-90-1981776, Fax: +66-2-2601532
E-mail: pansiri@g.swu.ac.th

decrease in VEGF expression levels in zebrafish[10]. In present study, the authors, therefore, focused on characterizing the expression patterns of neurotrophic factor genes (BDNF, NGF, NT-3, and VEGF) and the link between the clock genes (Per1, Cry1, Bmal1, and Rev-erb alpha) and these neurotrophic factor genes in rat hippocampus.

Material and Method

Animals

Adult Wistar rats (8 weeks of age; National Laboratory Animal Center of Mahidol University, Salaya, Thailand) were housed at least one week under light/dark (LD) regime with 12 hours (h) of light (lights on at 6:00 or Zeitgeber time (ZT) 0) and 12 h of darkness (lights off at 18:00) per day with access to food and tap water *ad libitum*. The study was performed in accordance with experimental protocols approved by the Animal Ethics Committee of the Faculty of Medicine, Srinakharinwirot University (under License No. 1/2553).

Tissue preparation

All animals were sacrificed at 6 h intervals throughout the daily cycle (n = 3 animals per time point). Animals in each group were rapidly decapitated under dim red light at ZT03, 09, 15, and 21. The hippocampus was dissected, quickly frozen in dry ice, and stored at -80°C until RNA isolation was performed.

RNA isolation, reverse transcription, and real-time PCR

The hippocampus was homogenized using sonics vibra-cell (Sonics & Materials INC., Newtown, CT, USA) and the total RNA was isolated using TRizol reagent (In vitro gen Life Technologies, Carlsbad, CA, USA). Two µg of each RNA sample was reverse-transcribed with High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA) according to the users manual, and real-time PCR reactions were set up using the SsoAdvanced™ SYBR® Green Supermix (Bio-Rad, California, USA) along with 1 ul of cDNA and the gene-specific primers (Bio-Rad, Hercules, CA, USA). Each sample was measured triplicately to ensure the accuracy of data on CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The reactions were performed under the following conditions: 95°C for 2 min as a polymerase activation step, 40 cycles of 95°C for 5 sec for denaturation, and 60°C for 30 sec for primer annealing and extension. The mRNA expression levels were analyzed by Bio-Rad CFX manager™ software version 1.3.1 (Hercules, CA) and quantification of relative mRNA expression was calculated using the comparative Ct method to normalize target gene mRNA to beta-actin mRNA (Actb). The details of primer assays are listed in Table 1.

Statistical analysis

All data were expressed as the mean from three animals $\pm$ SEM per time point. Statistical correlation was used to determine the relationship between the expression of clock genes and neurotrophic factor genes. One-way ANOVA analyzes with Tukey's post hoc test for specific comparisons were performed on all data. The $p<0.05$ was considered to be significant.

Results

Core clock genes expression in rat hippocampus

In adult rats, the real-time PCR analysis confirmed circadian rhythms in expression of entire

Table1. Details of primer assay for real-time PCR

Genes	Interrogated sequence		Translated protein	Amplicon length
	Assay ID.	Ref. sequence		
Per1	qRnoCED0008641	NM001034125.1	NP001029297.1	62
Cry1	qRnoCID0002158	NM198750.2	NP942045.2	119
Bmal1	qRnoCID0007909	NM_007489.4	NP_031515.1	85
Rev-erb alpha	qRnoCED0003337	NM145775.2	NP 665718.2	70
BDNF	qRnoCED0005012	rCT26305.0	rCP50611.0	90
NGF	qRnoCID0003911	NM001277055.1	NP001263984.1	103
NT-3	qRnoCID0053254	NM019248.2	NP062121.1	198
VEGF	qRnoCED0002159	NM031836.3	NP114024.2	120
Actb	qRnoCID0056984	NM031144.3	NP112406.1	74

studied clock genes in rat hippocampus. The Per1 and Rev-erb alpha expression profiles were roughly in the opposite phase to Cry1 and Bmal1 profiles (Fig. 1). On the basis of one-way ANOVA, the mRNA of Per1 (Fig. 1A) and Rev-erb alpha (Fig. 1C) showed a significant circadian expression with the highest peak at the light phase (ZT09; $p< 0.001$), whereas Cry1 (Fig. 1B) and Bmal1 (Fig. 1D) mRNAs displayed a significant circadian expression with the highest peak at the end of the night (ZT21; $p<0.05$ for Cry1 and $p<0.001$ for Bmal1).

Expression of neurotrophic factor genes in rat hippocampus

To determine whether the neurotrophin factor genes in hippocampus expressed in a circadian manner, the authors examined the expression of BDNF, NT-3, NGF, and VEGF in hippocampus at 6 h intervals by real-time PCR. The results showed that all selected neurotrophic factor mRNA, except NT-3, displayed a circadian rhythm in hippocampus (Fig. 2). The BDNF (Fig. 2A) mRNA levels were maximal at the dark phase (ZT21), with significantly higher values when compared to the other time points ($p<0.01$ for ZT03 and ZT15, and $p<0.05$ for ZT09). The NGF mRNA (Fig. 2B) also showed rhythmic expression with highest values at the dark phase ($p<0.05$ between ZT03 and ZT15). On the other hand, VEGF mRNA expression showed a robust circadian rhythmicity with peak at the light phase (Fig. 2D). The VEGF mRNA levels at ZT09 were significantly higher than other time points ($p<0.05$ for ZT03; $p<0.01$ for ZT15 and ZT21). The level of NT-3 mRNA in the hippocampus did not show significant variations over the time (Fig. 2C).

Relationship between clock genes and neurotrophic factor genes in rat hippocampus

The correlations between clock genes (Per1, Cry1, Bmal1, and Rev-erb alpha) and neurotrophic factor genes; BDNF, NT-3, NGF, and VEGF expression profiles in rat hippocampus were analyzed by linear correlation (Fig. 3). The expression of Per1 mRNA in the hippocampus showed no correlation to BDNF (r = -0.20; Fig. 3A), NGF (r = 0.27; Fig. 3B), NT-3 (r = 0.13; Fig. 3C) mRNA, while strong positive correlation to that of VEGF with r = 0.86 (Fig. 3D). The expression of Cry1 and Bmal1 also showed no correlation to NGF (r = 0.07; Fig. 3F and r = 0.22; Fig. 3J, respectively) and NT-3 (r = -0.05; Fig. 3G and r = 0.24; Fig. 3K, respectively). However, Cry1 and Bmal1 mRNA expression showed

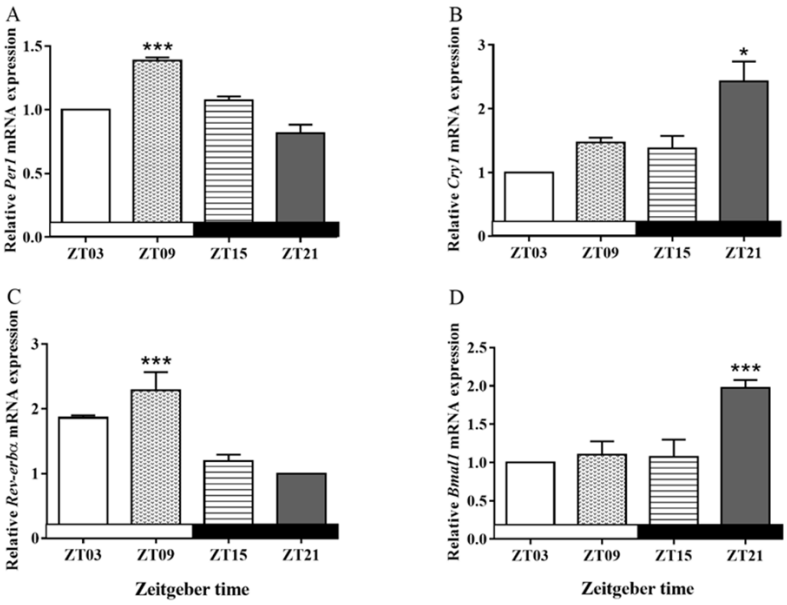

Fig. 1 Real-time PCR analysis of circadian mRNA expression profiles of clock genes in the hippocampus. Clock gene; Per1 (A), Cryl (B), Rev-erb alpha (C), and Bmal1 (D) mRNA expressed as relative values with respect to the mRNA amount at lowest time point (n = 3). White and black bars represented the light and the dark phase, respectively. Time sacrifice indicated as zeitgeber time (ZT). Bars represent mean ± SEM (n = 3, * $p<0.05$ and *** $p<0.001$).

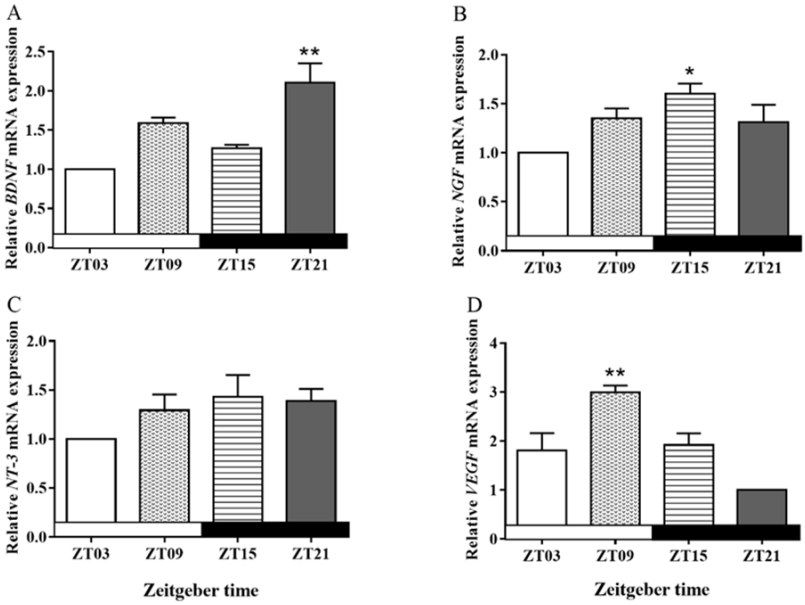

Fig. 2 Real-time PCR analysis of circadian mRNA expression profiles of neurotrophic factor genes in the hippocampus. The BDNF (A), NGF (B), NT-3 (C), and VEGF (D) mRNA expressed as relative values with respect to the mRNA amount at lowest time point (n = 3). White and black bars represented the light and the dark phase, respectively. Time sacrifice indicated as zeitgeber time (ZT). Bars represent mean ± SEM (n = 3; * p<0.05 and ** p<0.01).

positive correlation to BDNF with r = 0.73 (Fig. 3G) and r = 0.80 (Fig. 3K) as well as displayed the negative correlation to VEGF mRNA expression with r = -0.46 (Fig. 3H) and r = -0.55 (Fig. 3L), respectively. The expression of Rev-erb alpha mRNA showed no correlation to BDNF (r = -0.24; Fig. 3M), NGF (r = -0.15; Fig. 3N), and NT-3 (r = -0.08; Fig. 3O). Nevertheless, Rev-erb alpha mRNA expression was strong positively correlated to the expression of VEGF with r = 0.80 (Fig. 3P).

Discussion

The present study demonstrated the daily expression of four clock genes; Per1, Cry1, Bmal1, and Rev-erb alpha as well as four neurotrophic factor genes; BDNF, NT-3, NGF, and VEGF in rat hippocampus by real-time PCR. The mRNAs of Cry1 and Bmal1 are simultaneously elevated during late night that is in the opposite phase to the expression profiles of Per1 and Rev-erb alpha gene. The present finding indicates a common mechanism of transcriptional regulation of hippocampal clock genes in circadian manner corresponding to previously published data[3,11]. Besides clock gene expression, diurnal rhythms of BDNF mRNA and protein levels have been reported in the cerebellum, hippocampus, and cerebral cortex[12-14]. Consistently, herein, the authors found not only BDNF but also NGF and VEGF (but notably not of NT-3) genes display rhythmic expression patterns in the rat hippocampus. The BDNF mRNA expression is significantly high during the dark phase and low during the light phase in accordance with previous study[13,14]. In addition, the significant time difference between ZT03 and ZT15 of NGF mRNA expression was found. Thus, the expression of NGF mRNA seems to be a nocturnal pattern. However, it was not obvious throughout the day. Furthermore, the expression of VEGF is significantly higher at ZT09 which correlated with the expression of Per1 and Rev-erb alpha genes. Interestingly, correlation analysis revealed a strong relationship between all studies clock genes and VEGF. Moreover, BDNF also showed a good correlation to Cry1 and Bmal1 mRNA. Although there was a significant effect of time on expression of NGF mRNA, the correlation between the expression of NGF mRNA and all studies clock genes was not found. Remarkably, the neurotrophic factor genes and clock genes that showed a strong correlation would have the same expression pattern of daily circadian rhythm including; diurnal pattern, VEGF[15] with Per1 and Rev-erb alpha, and nocturnal pattern, BDNF[13,14] with Cry1 and Bmal1.

Currently, it is unknown what drives and gates

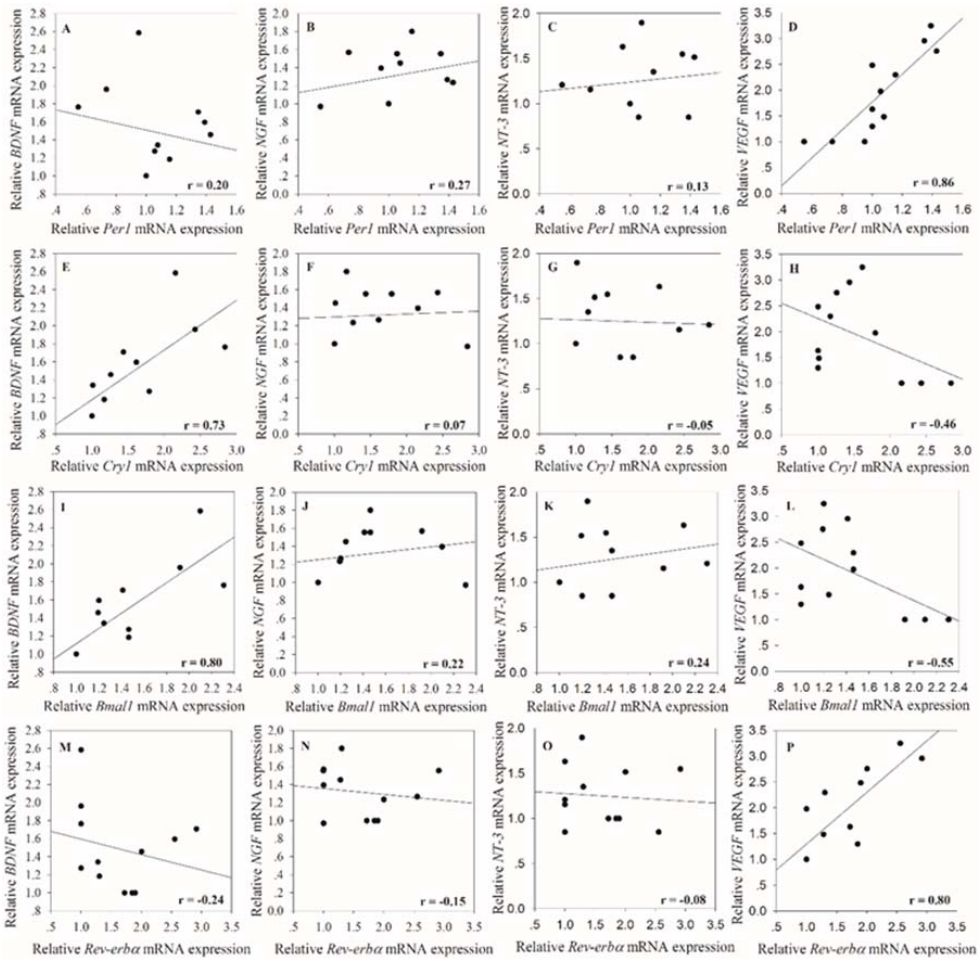

Fig. 3 Linear correlations between the expression pattern of clock genes (Per1; first row, Cry1; second row, Bmal1; third row, and Rev-erb alpha; fourth row) and neurotrophic factor genes (BDNF; first column, NGF; second column, NT-3; third column, and VEGF; fourth column) in the rat hippocampus. Values are gene expression levels as compared to minimum levels at ZT03. The correlation coefficients (r) are given inside the scatter plot.

circadian clock gene expression in the hippocampus. According to present results, VEGF or BDNF may be the possible candidate to be a factor drives hippocampal clock gene expression. Other researchers reported that both VEGF and BDNF contain clock responsive, E-box, sites in their gene promoter regions[15-17]. Hence, the relationship of clock gene and these neurotrophic factors should be under the terms of clock gene and clock-controlled genes. Previous study indicated that Bmal1 and Per2 could regulate circadian expression of VEGF mRNA[15,17,18]. In addition, it is reported that expression of the VEGF and BDNF genes were regulated by circadian rhythms[6,7,10,19]. Previous reports demonstrated that BDNF-expressing cell in the ventral SCN, the circadian pacemaker, had an important role in the regulation of circadian function in the SCN[8]. Moreover, the disturbance of circadian rhythm could induce the alteration of VEGF expression levels[10,18] showing that this neurotrophic factors expressions may be susceptible to an alteration of clock gene.

It is well established that neurotrophic factors, especially BDNF, are involved in neuronal plasticity such as learning and memory[20]. For example, exogenous BDNF facilitated the induction of long-term potentiation (LTP) in hippocampal slice while scavenging endogenous BDNF reduced the magnitude of hippocampal LTP[21]. Furthermore, administration of VEGF enhanced spatial memory, while inhibitors of VEGF-receptor impaired spatial memory[5,22]. Furthermore, the role of circadian rhythms on memory formation have been determined in intact animals by various behavioral paradigms[23,24], and clock gene-

deficient animals showed an impairment in hippocampus-dependent memory formation[11,25]. This is one possible way that clock gene associated with learning and memory in hippocampus may pass through the regulation of these neurotrophic factors. Given that it is hard to link molecular study to behavioral endpoints, further research is required to elucidate the explicit mechanisms and their precise role.

In conclusion, the results of the present study demonstrate that some neurotrophic factors expressed in hippocampus with a circadian manner are similar to the expression of clock gene. In this regard, the authors suggest that some neurotrophic factor genes, BDNF and VEGF, are the clock-controlled genes relating to specific clock genes. Therefore, further investigation of the molecular mechanisms linking cognitive processes and circadian clock in hippocampus could be necessary to clarify their functions.

What is already known on this topic?

The expression of circadian clock genes and BDNF mRNA has been reported in the rat SCN and hippocampus, but the time sacrifice and detection method are different from the present work.

What this study adds?

The present study is the first documentation of the relationship between the expression of four clock genes (Per1, Cry1, Bmal1, and Rev-erb alpha) and neurotrophic factor genes (BDNF, NGF, NT-3, and VEGF) in rat hippocampus throughout the day. The neurotrophic factors genes; BDNF and VEGF have been suggested to the clock-controlled genes in the rat hippocampus.

Acknowledgements

This work was supported by a Research fund of the Faculty of Medicine, Srinakharinwirot University (No. 031/2553) to PP and a Research Grant (DPG5780001) from Thailand Research Fund (TRF) and Mahidol University to PG.

Potential conflicts of interest

None.

References

1. Okamura H, Yamaguchi S, Yagita K. Molecular machinery of the circadian clock in mammals. Cell Tissue Res 2002; 309: 47-56.
2. Bozek K, Relogio A, Kielbasa SM, Heine M, Dame C, Kramer A, et al. Regulation of clock-controlled genes in mammals. PLoS One 2009; 4: e4882.
3. Pramong R, Wongchitrat P, Govitrapong P, Phansuwan-Pujito P. Development of Clock Genes Expression in Rat Hippocampus. J Med Assoc Thai 2015; 98 (Suppl 9): S123-9.
4. Conner JM, Franks KM, Titterness AK, Russell K, Merrill DA, Christie BR, et al. NGF is essential for hippocampal plasticity and learning. J Neurosci 2009; 29: 10883-9.
5. Pati S, Orsi SA, Moore AN, Dash PK. Intra-hippocampal administration of the VEGF receptor blocker PTK787/ZK222584 impairs long-term memory. Brain Res 2009; 1256: 85-91.
6. Allen GC, West JR, Chen WJ, Earnest DJ. Developmental alcohol exposure disrupts circadian regulation of BDNF in the rat suprachiasmatic nucleus. Neurotoxicol Teratol 2004; 26: 353-8.
7. Liang FQ, Walline R, Earnest DJ. Circadian rhythm of brain-derived neurotrophic factor in the rat suprachiasmatic nucleus. Neurosci Lett 1998; 242: 89-92.
8. Liang FQ, Allen G, Earnest D. Role of brain-derived neurotrophic factor in the circadian regulation of the suprachiasmatic pacemaker by light. J Neurosci 2000; 20: 2978-87.
9. Pizzio GA, Hainich EC, Plano SA, Ralph MR, Golombek DA. Nerve growth factor-induced circadian phase shifts and MAP kinase activation in the hamster suprachiasmatic nuclei. Eur J Neurosci 2005; 22: 665-71.
10. Jensen LD, Cao Z, Nakamura M, Yang Y, Brautigam L, Andersson P, et al. Opposing effects of circadian clock genes bmal1 and period2 in regulation of VEGF-dependent angiogenesis in developing zebrafish. Cell Rep 2012; 2: 231-41.
11. Jilg A, Lesny S, Peruzki N, Schwegler H, Selbach O, Dehghani F, et al. Temporal dynamics of mouse hippocampal clock gene expression support memory processing. Hippocampus 2010; 20: 377-88.
12. Katoh-Semba R, Tsuzuki M, Miyazaki N, Matsuda M, Nakagawa C, Ichisaka S, et al. A phase advance of the light-dark cycle stimulates production of BDNF, but not of other neurotrophins, in the adult rat cerebral cortex: association with the activation of CREB. J Neurochem 2008; 106: 2131-42.
13. Bova R, Micheli MR, Qualadrucci P, Zucconi GG. BDNF and trkB mRNAs oscillate in rat brain during the light-dark cycle. Brain Res Mol Brain Res 1998; 57: 321-4.
14. Berchtold NC, Oliff HS, Isackson P, Cotman CW.

Hippocampal BDNF mRNA shows a diurnal regulation, primarily in the exon III transcript. Brain Res Mol Brain Res 1999; 71: 11-22.
15. Koyanagi S, Kuramoto Y, Nakagawa H, Aramaki H, Ohdo S, Soeda S, et al. A molecular mechanism regulating circadian expression of vascular endothelial growth factor in tumor cells. Cancer Res 2003; 63: 7277-83.
16. Golini RS, Delgado SM, Navigatore Fonzo LS, Ponce IT, Lacoste MG, Anzulovich AC. Daily patterns of clock and cognition-related factors are modified in the hippocampus of vitamin A-deficient rats. Hippocampus 2012; 22: 1720-32.
17. Jensen LD, Cao Y. Clock controls angiogenesis. Cell Cycle 2013; 12: 405-8.
18. Bhatwadekar AD, Yan Y, Qi X, Thinschmidt JS, Neu MB, Li CS, et al. Per2 mutation recapitulates the vascular phenotype of diabetes in the retina and bone marrow. Diabetes 2013; 62: 273-82.
19. Fujita S, Koyama Y, Higashimoto M, Ono K, Ono T, Watanabe K, et al. Regulation of circadian rhythm of human vascular endothelial growth factor by circadian rhythm of hypoxia inducible factor-1α: implication for clinical use as anti-angiogenic therapy. Ann Cancer Res Therap 2010; 18: 28-36.
20. Yamada K, Mizuno M, Nabeshima T. Role for brain-derived neurotrophic factor in learning and memory. Life Sci 2002; 70: 735-44.
21. Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 1996; 381: 706-9.
22. Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, et al. VEGF links hippocampal activity with neurogenesis, learning and memory. Nat Genet 2004; 36: 827-35.
23. Eckel-Mahan KL, Storm DR. Circadian rhythms and memory: not so simple as cogs and gears. EMBO Rep 2009; 10: 584-91.
24. Smarr BL, Jennings KJ, Driscoll JR, Kriegsfeld LJ. A time to remember: the role of circadian clocks in learning and memory. Behav Neurosci 2014; 128: 283-303.
25. Kondratova AA, Dubrovsky YV, Antoch MP, Kondratov RV. Circadian clock proteins control adaptation to novel environment and memory formation. Aging (Albany NY) 2010; 2: 285-97.

ความสัมพันธ์ระหว่างจีนควบคุมเวลาและนิวโรโทรฟีคแฟกเตอร์ในสมองส่วนฮิปโปแคมปัสของหนู

รัชฎาภรณ์ ประมงค์, ปิยะรัตน์ โกวิทตรพงศ์, ปานสิริ พันธุ์สุวรรณ

ภูมิหลัง: สมองส่วนฮิปโปแคมปัสเป็นสมองส่วนสำคัญที่เกี่ยวข้องกับการเรียนรู้จดจำและมีการแสดงออกของจีนควบคุมเวลาเป็นจังหวะรอบวัน นอกจากนี้ยังพบนิวโรโทรฟีคแฟกเตอร์ที่สำคัญกับกระบวนการเรียนรู้ในสมองส่วนนี้ ซึ่งการแสดงออกของจีนควบคุมเวลาและนิวโรโทรฟีคแฟกเตอร์ในสมองส่วนฮิปโปแคมปัสน่าจะมีความจำเป็นต่อการเกิดระบบการสร้างความจำที่เหมาะสม

วัตถุประสงค์: เพื่อศึกษารูปแบบการแสดงออกของจีนนิวโรโทรฟีคแฟกเตอร์และหาความสัมพันธ์ระหว่างจีนควบคุมเวลาและนิวโรโทรฟีคแฟกเตอร์นี้ในสมองส่วนฮิปโปแคมปัสของหนูแรท

วัสดุและวิธีการ: ตรวจวัดปริมาณการแสดงออกของจีนควบคุมเวลา Per1 Cry1 Bmal1 และ Rev-erb alpha และจีนนิวโรโทรฟีคแฟกเตอร์ BDNF NGF NT-3 และ VEGF ในสมองส่วนฮิปโปแคมปัสของหนูแรททุก 6 ชั่วโมง โดยเทคนิค real-time PCR

ผลการศึกษา: มีการแสดงออกของจีน BDNF NGF และ VEGF เป็นจังหวะรอบวันเช่นเดียวกับจีนควบคุมเวลา และพบความสัมพันธ์ระหว่างการแสดงออกของจีนควบคุมเวลาที่ศึกษาทั้งหมดกับจีน VEGF และมีความสัมพันธ์ระหว่างการแสดงออกของ BDNF กับจีน Cry1 และ Bmal1 อีกด้วย

สรุป: มีความสัมพันธ์ระหว่างการแสดงออกของนิวโรโทรฟีคแฟกเตอร์ VEGF และ BDNF กับจีนควบคุมเวลาในเทอมของ clock-controlled จีน โดยสัมพันธ์กับจีนควบคุมเวลาแตกต่างกัน และเนื่องจากนิวโรโทรฟีคแฟกเตอร์นี้ มีความจำเป็นต่อการเรียนรู้จดจำในสมองส่วนฮิปโปแคมปัส ดังนั้นการศึกษาต่อไปถึงกลไกระดับโมเลกุลของ ความสัมพันธ์นี้น่าจะทำให้เข้าใจบทบาทของจังหวะรอบวันต่อกระบวนการเรียนรู้จดจำในสมองส่วนนี้ยิ่งขึ้น