Chinese Journal of Physiology 58(1): 19-26, 2015
DOI: 10.4077/CJP.2015.BAC248

Effects of Light-Dark Cycle on Hippocampal iNOS Expression and CREB Activation in Rats

Chia-Yu Hsieh[1, 2, *], Chia-Hsiu Hung[3, *], Yi-Hsuan Lee[4], Shu-Ting Wu[2], and Chaur-Jong Hu[2]

[1]*Division of Cell Physiology and Neuroscience, Graduate Institute of Medical Sciences Taipei Medical University, Taipei 11031*
[2]*Department of Neurology, Taipei Medical University-Shuang Ho Hospital, New Taipei City 23561*
[3]*Department of Pediatrics, Taipei Medical University Wan Fang Hospital, Taipei 11696*
and
[4]*Department and Institute of Physiology, National Yang-Ming University, Taipei 11221*
Taiwan, Republic of China

Abstract

Nitric oxide (NO) is one of the most important interneural signaling molecules mediating hippocampal functions for learning and memory. The diurnal expression of neuronal NO synthase (nNOS) has been well studied. However, the temporal profile and underlying mechanisms of inducible NOS (iNOS) expression in a normal photoperiod or in altered light-dark cycles remain unclear. We examined the temporal profile of iNOS expression in adult male Sprague-Dawley rats maintained in a 12h-light/12h-dark photoperiod (12L/12D), which were pre-synchronized for 7 days before the experiment. The protein and mRNA levels of iNOS in the cortex and hippocampus were measured to examine the photic influences on iNOS expression. The results showed rhythmically changes of the levels of iNOS mRNA and protein in the hippocampus, but not in the cerebral cortex. The iNOS mRNA levels peaked at Zeitgeber time (ZT) 6 and ZT22, and the protein levels peaked at ZT8 and ZT18. Notably, the peaks in iNOS mRNA and protein levels in the 12L/12D group were 10 to 12 h apart, and the rhythmic pattern was absent in the 24-h period of the darkness group. In addition, the level of the phosphorylated cAMP response element binding protein (phospho-CREB) was the highest at ZT18, prior to a peak in iNOS mRNA expression at ZT22. A phospho-CREB-iNOS signaling pathway was further confirmed by the interaction of phospho-CREB and the iNOS DNA in histone complexes isolated by chromatin immunoprecipitation at ZT18. In conclusion, the photoperiod affects the diurnal expression of iNOS and activation of CREB in the hippocampus, which provide clues for the possible impact of circadian changes in hippocampal functions.

Key Words: cAMP response element binding protein (CREB), circadian, hippocampus, iNOS, light dark cycle

Introduction

The light/dark cycling in the photoperiod acts as an environmental stimulus that entrains the circadian rhythm on a 24-h time course (29). The primary signal transduction pathway for generating the circadian rhythm is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (24, 34). Previous studies have shown a relationship between the photoperiod and cognitive flexibility and emotionality (17).

Corresponding author: Chaur-Jong Hu, M.D., Department of Neurology, Taipei Medical University-Shuang Ho Hospital, No. 291, Zhongzheng Rd., Zhonghe District, New Taipei City 23561, Taiwan, R.O.C. Tel: +886-2-22490088 ext. 8157, Fax: +886-2-2737275, Mobil: +886-970746908, E-mail: chaurjongh@tmu.edu.tw
*C.Y. Hsieh and C.H. Hung contribute to this work equally.
Received: October 2, 2013; Revised: April 28, 2014; Accepted: May 23, 2014.
 ISSN : 0304-4920. http://www.cps.org.tw

Constant light conditions decrease hippocampal neurogenesis and induce cognitive dysfunction, suggesting that the photoperiod is critical in maintaining hippocampal functions (10). In humans, chronic jet lag results in a disturbance of the photoperiod, and is characterized by marked circadian disarrangement and cognitive deficits, especially in working memories (5).

Nitric oxide (NO) in the hippocampus is involved in various physiological regulations and subsequent behavioral presentation, such as memory consolidation, which is an important component of cognitive functions (11). The enzymes that catalyze the production of NO belong to the nitric oxide synthase (NOS) family that includes the neuronal NOS (nNOS), the endothelial NOS (eNOS), and the inducible NOS (iNOS) (19). Light stimulation changes the brain physiology in which NO plays an important role. Under light stimulation, glutamate release is triggered and bound to N-methyl, D-aspartate (NMDA) and non-NMDA receptors on SCN neurons, triggering NO-cyclic GMP (cGMP)-dependent pathways (6). In addition, both light stimulation and NO activate guanylate cyclase (GC)-cGMP-cGMP-dependent kinase pathways (9). The light signal is associated with phosphorylation of transcription factors, including the cAMP response element binding (CREB) protein that is involved in circadian rhythm-related gene expression in the SCN (18, 33). The promoter regions of the *nNOS*, *eNOS* and *iNOS* genes contain the cAMP response element (CRE), suggesting that NOS expression may be transcriptionally regulated by CREB (12).

Studies have shown that nNOS activities in the SCN exhibit a diurnal cycle (1, 7, 9). Prolonged waking during the light phase is associated with an increase in iNOS expression (13). In adult rats, iNOS expression is associated with neurogenesis of the dentate gyrus, which is under the regulation of a circadian marker, corticosterone (27). However, details of temporal expression of iNOS over the entire photoperiod are not well understood, and the underlying molecular mechanism of iNOS expression that is affected by light-dark cycling also remains unclear. The aims of our study were, to evaluate the expression profile of iNOS throughout the photoperiod, and to examine the mechanisms that are implicated in the photic regulation of iNOS expression.

Materials and Methods

Subjects

Adult, male Sprague-Dawley rats (Biolasco Experimental Animal Institute, Taipei, Taiwan) were housed in groups of 3 to 4 animals. Food and water were made available. All animal protocols were approved by the Experimental Animal Review Committee at Taipei Medical University, and experiments were conducted according to the ethical and methodological guidelines for rhythm research on animals (28).

Time Course Setting

Rats were maintained in a 12-h-light/12-h-dark photoperiod (12L/12D) with lights on at 0800 and light off at 2000 for 7 days before the experiments were initiated. Rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital and euthanized every 2 h during a 24-h period. For the constant darkness condition (24D), rats were kept without light for 7 days, and were euthanized every 2 h under red light. The Zeitgeber time (ZT) system was used to indicate the time course. The transition from dark to light was set at 0800 with regard to ZT0. For the 24D condition, ZT was replaced by projected circadian time (pCT) to highlight the differences between the 12L/12D condition.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

iNOS expression was evaluated using semi-quantitative reverse transcription (RT) PCR. Total RNA was extracted from 20 to 30 mg of cerebral cortex and hippocampus tissues using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The complementary DNA (cDNA) was synthesized using 1 μg total RNA as the template by ImProm® II Reverse-transcription Kit (Promega, Madison, WI, USA). PCR was conducted by use of the Platinum Taq DNA Polymerase Kit I (Invitrogen). The PCR conditions of iNOS cDNA were denaturation at 94°C for 30 s, annealing at 55°C for 40 s, and extension at 70°C for 30 s. The cDNA of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was PCR-amplified in parallel as an internal control. Primers used for iNOS gene amplification were 5'-CCTTGTTCAGCTACGCCTTC-3' (forward) and 5'-CATGGTGAACACGTTCTTGG-3' (reverse), and those used for the GAPDH were 5'-ATGGTCAACCCCACCGTGT-3' (forward) and 5'-CGTGTGAAGTCACCACCCT-3' (reverse). The PCR products were separated in a 2% agarose gel by electrophoresis, and were stained with ethidium bromide. The gels were digitally photographed using the Image System (Fotodyne, Hartland, WI, USA), and the bands were quantified using NIH Image J1.37 software (National Institutes of Health, Bethesda, MD, USA).

Western Blot Analysis

Hippocampus samples, about 20 to 30 mg, were frozen in liquid nitrogen, and were homogenized twice

in Golden lysis buffer containing a protease inhibitor cocktail (BioMan, Taipei, Taiwan, ROC) and a Ser/Thr phosphatase inhibitor cocktail (BioMan, Taipei, Taiwan). For demonstrating the efficiency of the iNOS antibody, we used a protein lysate prepared from RAW 264.7 cells (ATCC, Manassas, VA, USA) after treatment of lipopolysaccharide (LPS) as the positive control in the 24D experiment. The RAW 264.7 cells were cultured in DMEM medium with 10% fetal bovine serum, penicillin (50 unit/ml) and streptomycin sulfate (50 μg/ml), and were incubated in a humidified atmosphere with 5% CO_2 at 37°C. The cells grew to a density of 4×10^5 cells/well in 6-well dishes. The cells were then treated with 100 μg/ml LPS (Sigma-Aldrich, St. Louis, MO, USA) for 24 h, and protein lysate was extracted for Western blot analysis. A total of 60 μg protein from each sample was separated electrophoretically in a 9% acrylamide gel by SDS-PAGE at 100 V for 2.5 h and was subsequently transferred onto a PVDF membrane (BioTrace, Pall, FL, USA). To detect iNOS, CREB and Ser-133-phosphorylated CREB (phospho-CREB), the membranes were incubated in a blocking buffer, followed by incubation with a mouse anti-iNOS monoclonal antibody (1:300; BD Transduction Laboratories, San Jose, CA, USA), a rabbit polyclonal anti-CREB antibody (1:1000; Millipore, Billerica, MC, USA), or a rabbit polyclonal anti-phospho-CREB antibody (1:1000; Millipore) overnight at 4°C. After washing, the membranes were treated with HRP-conjugated secondary antibodies, including goat anti-mouse-IgG (1:8000) or anti-rabbit-IgG (1:5000) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The β-actin protein served as an internal control using a mouse monoclonal anti-β-actin antibody (1:10000; Abcam, San Francisco, CA, USA). The protein bands were visualized using the Western Lightning Enhanced Chemiluminescence Substrate kit (Perkin Elmer, MA, USA). Blot images were digitized by the BioSpectrum AC Imaging System (UVP, Upland, CA, USA), and bands were quantified by the UVP Vision Works software.

Chromatin Immunoprecipitation Assay

A chromatin immunoprecipitation (ChIP) assay was used to survey the binding status between phospho-CREB and the iNOS gene promoter at ZT0, ZT2, ZT4, ZT6, ZT8, ZT12, ZT14, ZT16, ZT18, ZT20 and ZT22 (14). Approximately 50 mg of hippocampaus tissues was homogenized and cross-linked in 1% formaldehyde with 0.5 mM PMSF (Sigma). The cross-linking reaction was stopped by 0.125 M glycine, and samples were centrifuged at 1,000 rpm, 4°C for 5 min. After decanting, the pellets were resuspended in 1 ml cold swelling buffer (5 mM PIPES, 0.5% IPGEAL, 10X protease inhibitor and 0.5 mM PMSF), then were centrifuged. The pellets obtained were resuspended in 1 ml sonication buffer (10 mM, EDTA, 50 mM Tris-HCl, 0.5 mM PMSF and 1X Bioman protease inhibitor), and then sonicated on ice to shear the DNA using an ultrasound sonicator (Microson, New Town, CT, USA). To demonstrate the presence of phospho-CREB in histone complexes, 2 μg rabbit polyclonal anti-phospho-CREB (Ser-133) antibody (Millipore) was added, and the samples were incubated overnight at 4°C on a rotating wheel. Samples with no added antibody, or with the use of normal rabbit IgG served as negative controls. Precipitation was initiated by adding 30 μl of a salmon-sperm DNA and protein A agarose slurry (Millipore). Antibody/histone complexes were collected, and sequentially washed and centrifuged using low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), LiCl buffer (0.25 M LiCl, 1% IGEPEL-CA300, 1% deoxycholic acid, 1 mM EDTA, 1 mM Tris, pH 8.1), and TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The pelleted protein A agarose/antibody/chromatin complexes were resuspended in 200 μl freshly prepared elution buffer (1% SDS and 100mM $NaHCO_3$), and complexes were dissociated by adding 8 μl 5 M NaCl and heating at 65°C for 4 h. Proteins were digested by adding 0.8 μl 10 mg/ml proteinase K (Invitrogen), 4 μl 0.5 M EDTA, and 8 μl of 1 M Tris-HCl, pH 6.5, and heating at 45°C for 1 h. DNA was isolated from digested products using the QIAquick DNA spin column (Qiagen, Chatsworth, CA, USA). The iNOS promoter DNA was amplified using 0.5 to 1 μL DNA template, the primers were 5'-ACCTGCTG-AACTATCTCACCAAC-3' (forward), and 5'-ACA-CCAAGTAAGAGTCACCCAAA-3' (reverse). PCR consisted of 35 cycles with denaturing at 94°C for 30 s, annealing at 55°C for 40 s, and extension at 70°C for 30 s. The PCR products were analyzed by agarose gel electrophoresis. The gel images were collected using the Image System (Fotodyne), and quantified by the NIH Image J1.37 software.

Statistical Analysis

One way analysis of variance (ANOVA) followed by *post-hoc* tests was performed for statistic analysis. *P*-value less than 0.05 indicated statistic significance. All experiments were performed using three animals (n = 3). The data in the figures are expressed as mean and standard error of the mean.

Results

Analysis of iNOS mRNA expression in the cerebral cortex and the hippocampus is shown in Fig.

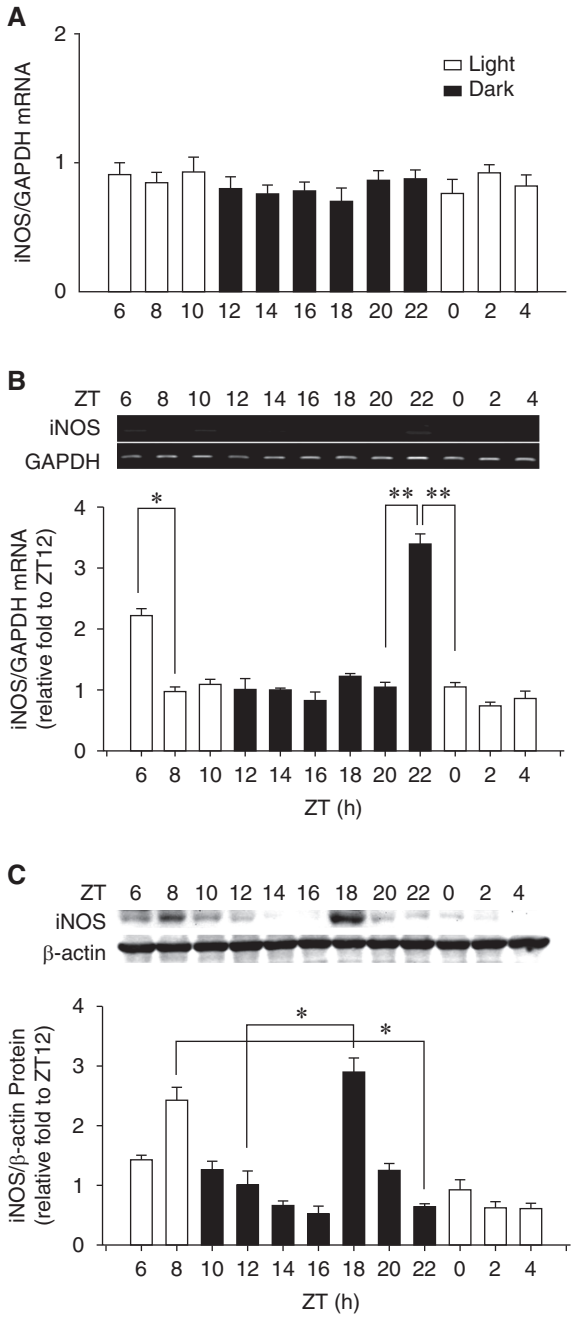

Fig. 1. Expression of inducible NOS (iNOS) mRNA and protein in the brain of adult male Sprague-Dawley rats during the 12L/12D cycle. Open bars and black bars indicate the mean ± standard error at the time points in the light phase and dark phase, respectively (n = 3; *$P < 0.05$). (A and B) RT-PCR analysis of iNOS mRNA expressed in (A) the cerebral cortex and (B) the hippocampus. (C) Western blot analysis of iNOS protein expression in the hippocampus.

1A. To evaluate iNOS expression throughout the photoperiod, iNOS mRNA expression in the cerebral cortex and hippocampus was examined. RT-PCR analysis showed that the iNOS mRNA expression in

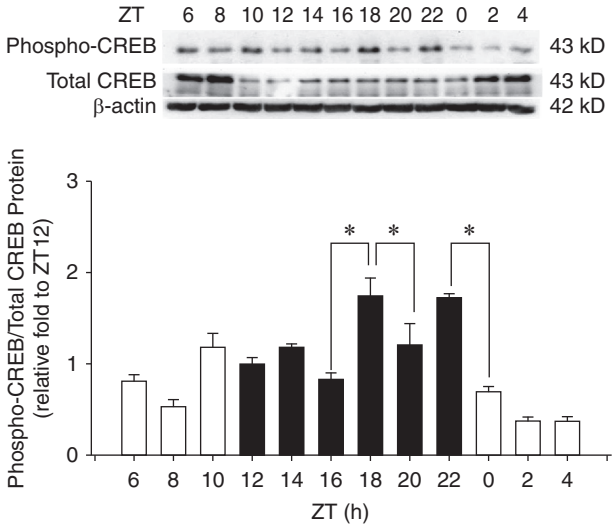

Fig. 2. Western blot analysis of total CREB and phospho-CREB protein levels in the hippocampus of adult male Sprague-Dawley rats during the 12L/12D cycle. Open bars and black bars indicate the mean ± standard error at the time points in the light phase and dark phase, respectively (n = 3; *$P < 0.05$). The data represent fold increases of phospho-CREB abundance relative to the total level of CREB proteins at each respective time point.

the hippocampus was significantly higher at ZT16, compared with that at ZT4 ($P < 0.05$; Fig. 1A). In contrast, no significant differences in the level of iNOS mRNA expression were noted among the cerebral cortex samples.

Temporal profile of iNOS mRNA and protein levels in the hippocampus are shown in Figs. 1B and 1C. To survey the temporal profile of diurnal iNOS expression, hippocampal tissues were dissected from rat brains every 2 h from the onset of light (ZT0). After the end of the light period (Z12), rats were euthanized, and tissue samples were collected by dissecting under red light to avoid light stimuli. Expression of iNOS mRNA was determined by comparing the RT-PCR data of all the time points with that of ZT12. The peaks of mRNA expression during the light phase at ZT6 and the dark phase at ZT22 ($P < 0.05$) are shown in Fig. 1B. Western blotting analysis of the temporal expression profile showed peaks of protein expression at ZT8 and ZT18, as compared with it at ZT12 (Fig. 1C; $P < 0.05$).

Results of CREB phosphorylation and CREB binding to iNOS promoter during the photoperiod are shown in Figs. 2 and 3. We further explored the potential involvement of CREB in the regulation of iNOS expression. Western blot analysis revealed a maximum level of phospho-CREB during the dark phase at ZT18 ($P < 0.05$; Fig. 2), and a second peak was observed during the dark phase at ZT22 ($P < 0.05$). The level of total CREB varied during the photoperiod,

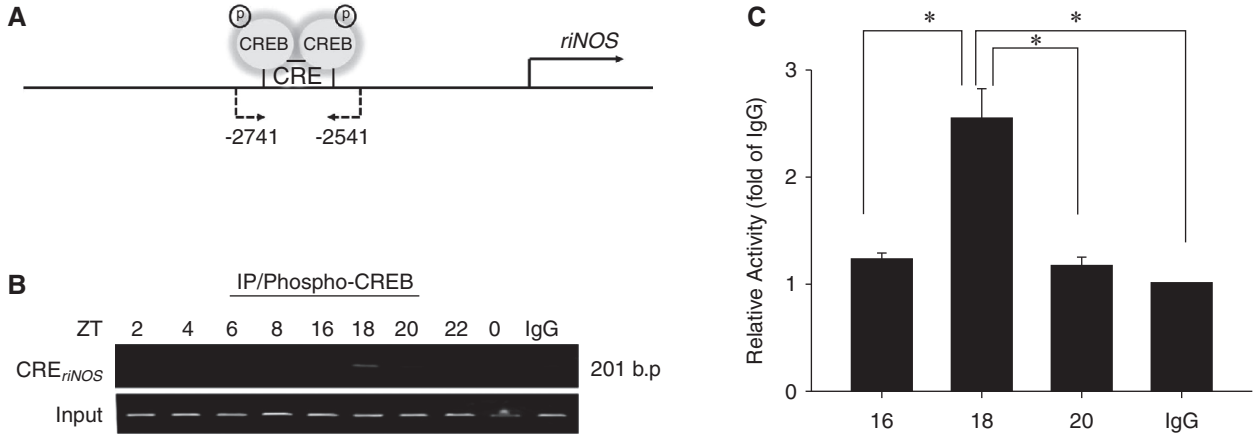

Fig. 3. Phospho-CREB binds to the cAMP response element on the iNOS promoter (CRE_{riNOS}) in the genomic DNA of the hippocampus of adult male Sprague-Dawley rats at ZT18 during the 12L/12D cycle. (A) Schematic diagram of the rat *iNOS* promoter: the blank box represents the putative CRE_{riNOS} and the dotted arrows indicate the nucleotide positions of the 5'- and 3'-end of the promoter that serve as priming sites for the chromatin-immunoprecipitation analysis. (B and C) PCR analysis of histone complexes at (B) ZT0 to ZT8 and (C) ZT16 to ZT22. Black bars represent the mean ± standard error relative to that of the normal rabbit IgG control at each respective time point (n = 3; $*P < 0.05$).

with higher levels observed during the light phase. A temporal relationship was observed between iNOS expression and CREB phosphorylation, which represents CREB activation, and which should occur before the peak in mRNA expression at ZT22. Western blot analysis data showed elevated levels of phospho-CREB at ZT16, ZT18, and ZT20 (Fig. 2). The ChIP assay results further showed a maximum level of phospho-CREB binding to the iNOS gene promoter at ZT18 ($P < 0.05$; Fig. 3).

Complete darkness failed to induce diurnal variation of iNOS and phospho-CREB, as shown in Fig. 4. Because of the potential relationship between iNOS expression and CREB phosphorylation, especially in the dark phase, we examined whether their diurnal variations observed in the light-dark cycle also existed when the animals were subjected to complete 24 h darkness (24D). Analysis of hippocampus samples from the 24D treatment group showed low levels of both iNOS mRNA and protein levels throughout the 24-h period (Fig. 4, A and B). CREB phosphorylation in the hippocampus also failed to show any significant expression changes or patterns under the 24D condition (Fig. 4, B and C, respectively).

Discussion

Previous studies have documented the association between circadian rhythm, which is entrained by the light-dark cycling of the photoperiod, and cognitive function (10, 30). Changes in gene expression in the hippocampus are responsible for memory formation and consolidation (4, 21, 25). The aims of our study were to determine the effects of photoperiod on hippocampal iNOS mRNA expression, and to examine the role of phospho-CREB in the regulation of iNOS mRNA expression. We found two iNOS mRNA/protein expression peaks, one peak occurring during light phase, and another in the dark phase. The peaks of mRNA and protein expression were both approximately 10 to 12 h apart, implicating that increases of iNOS protein were the result of increased levels of iNOS mRNA. This phenomenon disappeared in constant darkness, as evidenced by disappearance of iNOS mRNA and protein expression oscillation in the 24D group. These findings might suggest that the light-dark cycling during the photoperiod affects iNOS mRNA expression in the hippocampus. Moreover, the results provide a line of evidence for the impact of the photoperiod on gene expression in the brain.

Light stimuli have been shown to enhance the interaction of CREB and CRE in the SCN. Oscillated CREB phosphorylation in the hippocampus, and the maximum levels of phospho-CREB have been observed in the day time even during the free-running condition (8, 16). However, activation of CREB in the pineal gland during the dark phase has also been reported in the signaling pathway involved in production of melatonin in the early evening (15). The detailed mechanisms of CREB activation under regular light-dark cycle and free-running condition might need further exploration. Anyhow, activation of CREB is possibly an upstream event of iNOS mRNA expression in the light-dark cycle. To understand the role of CREB in the regulation of iNOS mRNA and protein expression, the temporal pattern of phospho-CREB and total CREB in the light-dark cycle was surveyed. The results showed that phospho-CREB

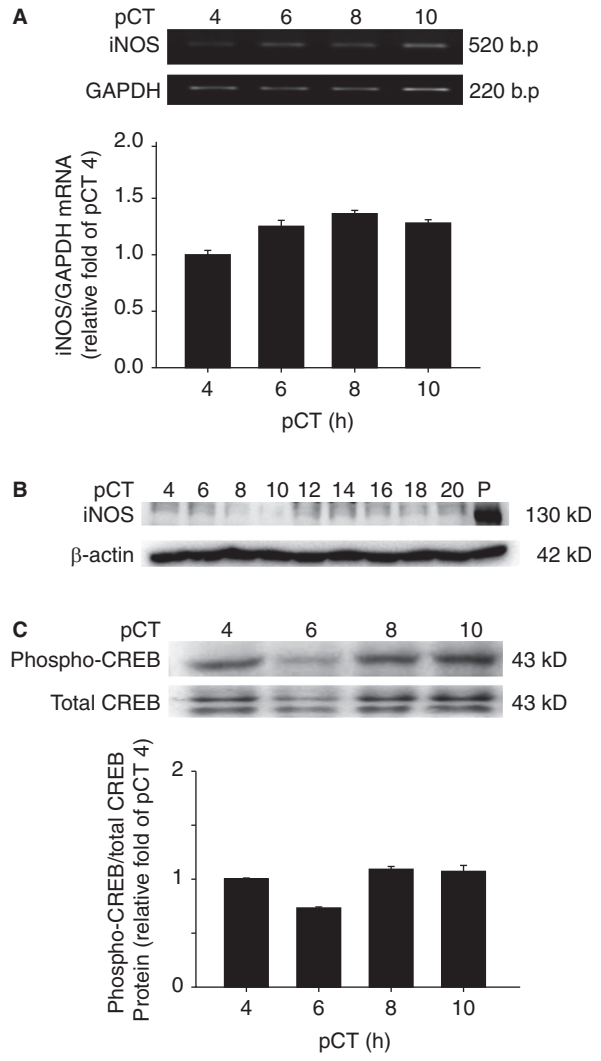

Fig. 4. Expression of iNOS mRNA in the hippocampus of adult male Sprague-Dawley rats kept in constant darkness (24D; n = 3; *$P < 0.05$). RT-PCR analysis was performed using total RNA from hippocampal tissue samples that were harvested at projected circadian time (pCT) 4, pCT6, pCT8 and pCT10. Black bars indicate mean ± standard error of iNOS mRNA expression. (B) Western blot analysis of iNOS protein expression in the hippocampus during the 24D condition. Data for the β-actin protein expression control are also shown. Protein harvested from RAW 264.7 cells treated with 100 μg/mL lipopolysaccharide (P) was used as a positive control for iNOS protein expression. (C) Western blot analysis of total CREB and phospho-CREB protein levels in the hippocampus of rats during the 24D condition. Black bars indicate mean ± standard error of fold increases of phospho-CREB/CREB ratios at each respective time point.

increased at ZT18, relative to total CREB. The interaction between phospho-CREB and the CRE on the iNOS promoter was also confirmed at ZT18 by ChIP assays. These findings suggest that phospho-CREB up-regulates iNOS mRNA expression in the dark phase, but that CREB played no role in iNOS mRNA and protein expression in the light phase. In the 24D experiments, oscillation of both iNOS mRNA expression and CREB activation disappeared, implicating that iNOS mRNA expression in the hippocampus is influenced by the light-dark cycle, and that phospho-CREB is involved. Although other diurnal physiological rhythms may have been disrupted during the extended dark period, we observed that the diurnal expression of iNOS protein was indeed abolished within a 24-h darkness period. Therefore, further studies of the photoperiod-mediated activation of CREB in the hippocampus are warranted. On the other hand, we showed that CREB played no role in iNOS mRNA and protein expression in the light phase. The mechanism of iNOS expression regulation remains to be further investigated. previous researches have documented that iNOS enzyme activities in hippocampus increased during the light phase and melatonin is considered to be involved (22). Melatonin is a circadian hormone which is suppressed by light under the regulation of SCN (20). Melatonin has been found to inhibit NOS synthesis. Therefore, it is speculated that increased iNOS mRNA expression after the impulse of light during the constant darkness condition could be melatonin-related (2, 23).

There are limitations to the findings of our study. Firstly, the total CREB expression was low during the dark phase and the temporal profile of total CREB showed greater fluctuations than the phospho-CREB profile. This phenomenon may have caused higher phospho-CREB/CREB ratios and challenges the interpretation of increased phospho-CREB (Fig. 2). However, the absolute phospho-CREB level did increase at ZT18 in our experiments and the phospho-CREB/CREB ratios reflect the activation of CREB more significantly. Further studies by inhibition of phospho-CREB might be warranted. Secondly, the physiological consequences of photoperiod-mediated hippocampal iNOS expression are unclear. An increase of iNOS expression has been used as a marker for reactions to sustained stress (26), and the hippocampus is a brain region responsible for stress reaction, emotional control and cognitive functions (3, 31, 32, 35). Additional studies to further clarify the role of iNOS expression in these physiological contexts are needed. Furthermore, we did not examine the activation of CREB or iNOS expression in the hippocampus of rats during the initial period of darkness. It is possible that slight differences among the free running periods of the rats might have been amplified by the 7-day period in constant darkness, which might have masked some diurnal rhythms due to increased variance from pCT sampling time points, compared with actual diurnal activity levels. Recording the circadian activities of

rats in constant darkness should be considered in further investigations.

Acknowledgments

We thank Dr. Ding-I Yang for his assistance in data analysis and manuscript preparation. This study was supported by grants awarded by the National Science Council, Taiwan (97-2314-B-038-010, 97-2314-B-038-026-MY2, 100-2314-B-038-014) and by Taipei Medical University Wan Fang Hospital (94TMU-WFH-217).

References

1. Agostino, P.V., Ferreyra, G.A., Murad, A.D., Watanabe, Y. and Golombek, D.A. Diurnal, circadian and photic regulation of calcium/calmodulin-dependent kinase II and neuronal nitric oxide synthase in the hamster suprachiasmatic nuclei. *Neurochem. Int.* 44: 617-625, 2004.
2. Camacho M.E., Carrion M.D., Lopez-Cara L.C., Entrena A., Gallo MS., Espinosa A., Escames G. and Acuña-Castroviejo D. Melatonin synthetic analogs as nitric oxide synthase inhibitors. *Mini Rev. Med. Chem*. 12: 600-617, 2012.
3. Campisi, J., Leem, T.H., Greenwood, B.N., Hansen, M.K., Moraska, A., Higgins, K., Smith, T.P. and Fleshner, M. Habitual physical activity facilitates stress-induced HSP72 induction in brain, peripheral, and immune tissues. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 84: R520-R530, 2003.
4. Cheng, R.K., Williams, C.L. and Meck, W.H. Neurophysiological mechanisms of sleep-dependent memory consolidation and its facilitation by prenatal choline supplementation. *Chinese J. Physiol.* 52: 223-235, 2009.
5. Cho, K. Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. *Nat. Neurosci.* 4: 567-568, 2001.
6. Colwell, C.S. and Menaker, M. NMDA as well non-NMDA receptor antagonists can prevent the phase-shifting effects of light on the circadian system of the golden hamster. *J. Biol. Rhythms* 7: 125-136, 1992.
7. Ding, J.M., Chen, D., Weber, E.T., Faiman, L.E., Rea, M.A. and Gillette, M.U. Resetting the biological clock: mediation of nocturnal circadian shifts by glutamate and NO. *Science* 266: 1713-1717, 1994.
8. Eckel-Mahan, K.L., Phan, T., Han, S., Wang, H., Chan, G.C., Scheiner, Z.S. and Storm, D.R. Circadian oscillation of hippocampal MAPK activity and cAMP: implications for memory persistence. *Nat. Neurosci.* 11: 1074-1082, 2008.
9. Ferreyra, G.A., Cammarota, M.P. and Golombek, D.A. Photic control of nitric oxide synthase activity in the hamster suprachiasmatic nuclei. *Brain Res.* 797: 190-196, 1998.
10. Fujioka, A., Fujioka, T., Tsuruta, R., Izumi, T., Kasaoka, S. and Maekawa, T. Effects of a constant light environment on hippocampal neurogenesis and memory in mice. *Neurosci. Lett.* 488: 41-44, 2011.
11. Furini, C.R., Rossato, J.I., Bitencourt, L.L., Medina, J.H., Izquierdo, I. and Cammarota, M. β-adrenergic receptors link NO/sGC/PKG signaling to BDNF expression during the consolidation of object recognition long-term memory. *Hippocampus* 20: 672-683, 2010.
12. Galea, E. and Feinstein, D.L. Regulation of the expression of the inflammatory nitric oxide synthase (NOS2) by cyclic AMP. *FASEB J.* 13: 2125-2137, 1999.
13. Henna-Kaisa, M.W. and Tarja, P.H. Inducible nitric oxide synthase and AMP-activated protein kinase in basal forebrain during prolonged waking. *NeuroReport* 20: 97-101, 2009.
14. Huang, H.S., Matevossian, A., Jiang, Y. and Akbarian, S. Chromatin immunoprecipitation in postmortem brain. *J. Neurosci. Methods* 156: 284-292, 2006.
15. Jockers, R., Maurice, P., Boutin, J.A. and Delagrange, P. Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Brit. J. Pharmacol.* 154: 1182-1195, 2008.
16. Kako, K., Banasik, M., Lee, K. and Ishida, N. Regulation of cAMP response element binding protein (CREB) binding in the mammalian clock pacemaker by light but not a circadian clock. *Brain Res. Mol. Brain Res.* 44: 39-45, 1997.
17. Karatsoreos, I.N., Bhagat, S., Bloss, E.B., Morrison, J.H. and McEwen, B.S. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci. USA* 108: 1657-1662, 2011.
18. Katoh-Semba, R., Tsuzuki, M., Miyazaki, N., Matsuda, M., Nakagawa, C., Ichisaka, S., Sudo, K., Kitajima, S., Hamatake, M., Hata, Y. and Nagata, K. 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.* 106: 2131-2142, 2008.
19. Knowles, R.G. and Moncada, S. Nitric oxide synthases in mammals. *Biochem. J.* 2: 249-258, 1994.
20. Koh, P.O. Melatonin regulates nitric oxide synthase expression in ischemic brain injury. *J. Vet. Med. Sci.* 70: 747-750, 2008.
21. Liang, K.C. Involvement of the amygdala and its connected structures in formation and expression of inhibitory avoidance memory: issues and implications. *Chinese J. Physiol.* 52: 196-214, 2009.
22. Marcola, M., da Silveira Cruz-Machado, S., Fernandes, P.A., Monteiro, A.W., Markus, R.P. and Tamura, E.K. Endothelial cell adhesiveness is a function of environmental lighting and melatonin level. *J. Pineal Res.* 54: 162-169, 2012.
23. Maronde, E., Schomerus, C., Stehle, J.H. and Korf, H.W. Control of CREB phosphorylation and its role for induction of melatonin synthesis in rat pinealocytes. *Biol. Cell* 89: 505-511, 1997.
24. Moore, R.Y. and Eichler, V.B. Loss of a circadian adrenal corticosterone rhythm following suprachaismatic lesions in the rat. *Brain Rev.* 42: 201-206, 1972.
25. Morgado-Bernal, I. Learning and memory consolidation: linking molecular and behavioral data. *Neuroscience* 10: 12-19, 2011.
26. Mutlu, G., Ulak, A., Laugeray, C. and Belzung, O. Effects of neuronal and inducible NOS inhibitor 1-[2-(trifluoromethyl) phenyl] imidazole (TRIM) in unpredictable chronic mild stress procedure in mice. *Pharmacol. Biochem. Behav.* 92: 82-87, 2009.
27. Pinnock, S.B., Balendra, R., Chan, M., Hunt, L.T., Turner-Stokes, T. and Herbert, J. Interactions between nitric oxide and corticosterone in the regulation of progenitor cell proliferation in the dentate gyrus of the adult rat. *Neuropsychopharmacology* 32: 493-504, 2007.
28. Portaluppi, F., Smolensky, M.H. and Touitou, Y. Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27: 1911-1929, 2010.
29. Roenneberg, T. and Merrow, M. Circadian clocks - the fall and rise of physiology. *Nat. Rev. Mol. Cell Biol.* 6: 965-971, 2005.
30. Roedel, A., Storch, C., Holsboer, F. and Ohl, F. Effects of light or dark phase testing on behavioural and cognitive performance in DBA mice. *Lab. Anim.* 40: 371-381, 2006.
31. Shao, S.H., Shi, S.S., Li, Z.L., Zhao, M.S., Xie, S.Y. and Pan, F. Aging effects on the BDNF mRNA and TrkB mRNA expression of the hippocampus in different durations of stress. *Chinese J. Physiol.* 53: 285-293, 2010.
32. Sarbadhikari, S.N. and Saha, A.K. Moderate exercise and chronic stress producecounteractive effects on different areas of the brain by acting through various neurotransmitter receptor subtypes: a hypothesis. *Theor. Biol. Med. Model* 9: 3-33, 2006.
33. Shearman, L.P., Sriram, S., Weaver, D.R., Maywood, E.S., Chaves,

I., Zheng, B., Kume, K., Lee, C.C., van der Horst, G.T., Hastings, M.H. and Reppert, S.M. Interacting molecular loops in the mammalian circadian clock. *Science* 288: 1013-1019, 2000.

34. Stephan, F.K. and Zucker, I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc. Natl. Acad. Sci. USA* 69: 1583-1586, 1972.
35. Wang, J., Chen, X., An, G., Zhang, N. and Ma, Q. Effects of exercise on stress-induced changes of norepinephrine and serotonin in rat hip-pocampus. *Chinese J. Physiol.* 56: 245-252, 2013.