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The gut–brain peptide neuromedin U is involved in the mammalian circadian oscillator system

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Abstract

Immunohistochemical analysis revealed the presence of a gut–brain peptide, neuromedin U (NMU), in the suprachiasmatic nucleus (SCN), which is the site of the master circadian oscillator. The expression of NMU mRNA exhibited a circadian rhythm, with the peak expression in the SCN occurring at CT4–8 h. The two NMU-binding receptors (NMU-R1 and NMU-R2) were also expressed in the SCN, but their phase angles were different. Intracerebroventricular injection (ICV) of NMU induced the expression of Fos protein in the SCN cells and caused a phase-dependent phase shift of the circadian locomotor activity rhythm. The magnitude of the phase shift was dose dependent. This NMU-induced phase shift was of the nonphotic type. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed increases in the expression in the SCN of immediate early genes, such as *c-fos*, *NGFI-A*, *NGFI-B*, and *JunB*. Furthermore, ICV injection of NMU increased the expression of *Per1*, but not *Per2*, in the SCN. These results indicate that NMU may play some important role in the circadian oscillator by exerting an autocrine or paracrine action in the SCN.

Keywords: Circadian rhythm; Fos; Neuromedin U; Per; Phase shift; Suprachiasmatic nucleus

Neuromedin U (NMU), which was originally identified in the porcine spinal cord, was so named because of its potent uterine-contraction-inducing activity [1]. NMU is a highly conserved gut–brain peptide present in a wide range of animals from mammals to amphibians, indicating that it plays an essential role in neuroendocrine function [2,3]. NMU is distributed widely in the gastrointestinal tract, pituitary gland, and central nervous system [4–6]. The peripheral activities of NMU include smooth muscle contraction [1], blood pressure elevation [7], and modification of intestinal ion transport [8], whereas centrally, NMU suppresses feeding [9,10] and induces the release of stress-mediating molecules such as adrenocorticotropic hormone (ACTH) and corticosterone [11,12].

Two homologous orphan G-protein-coupled receptors have been identified as NMU receptors: NMU-R1 and NMU-R2 [9,10,13]. These two receptors are expressed in numerous tissues, although NMU-R1 is found primarily in the periphery whereas NMU-R2 is expressed preferentially in the central nervous system, and especially in the hypothalamic paraventricular nucleus (PVN), the wall of the third ventricle, and the CA1 region of the hippocampus [9,13]. The widespread distribution of these two NMU receptors suggests additional functions of NMU in the central nervous system.

Recently, we and other groups found that NMU exists in the hypothalamic SCN [9], indicating that NMU might be involved in the regulation of circadian rhythm, since it is well known that the SCN functions as a biological clock [14–16]. In the study presented here,

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the aim was to determine whether or not NMU is involved in the circadian oscillator system.

Materials and methods

Animals and intracerebroventricular injection of NMU. Male Wistar rats weighing 280–300 g were maintained in individual cages under controlled temperature (21–23 °C) and light (light on 07:00–19:00 h) conditions with ad libitum access to food and water. Intracerebroventricular (ICV) cannulae were implanted into the lateral cerebral ventricles by a method that has been described previously [17]. Proper placement of the cannulae was verified by dye administration at the end of the experiments. Each rat was sham-injected with saline prior to the study, and weighed and handled daily. Only animals demonstrating progressive weight gain following surgery were used for subsequent experiments. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

Immunofluorescence staining for NMU and Fos. Immunohistochemical analyses for NMU were performed following injection of colchicine (100 μg/rat) into the lateral ventricle of rats through implanted cannulae, to increase immunostaining sensitivity, 30 h before perfusion with 2% paraformaldehyde. All rats were anesthetized by an intraperitoneal injection of pentobarbital, perfused transcardially with 100 ml of 0.1 M phosphate buffer (pH 7.4) containing 100 U heparin, and then with 150 ml fixative containing 2% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed, placed in fixative for 2 days at 4 °C, and then transferred to 0.1 M phosphate buffer containing 20% sucrose. Sections were cut at a thickness of 12 μm with a cryostat at a temperature of −20 °C. The sections were incubated for 2 days with anti-NMU antiserum (a polyclonal antibody raised in rabbits against rat NMU) at 4 °C, then with Alexa-546-labeled goat anti-rabbit IgG antibody (Molecular Probes, dilution 1:400) for 2 h. Samples were observed under an Olympus AX-70 fluorescence microscope (Olympus, Tokyo, Japan).

To examine the effect of ICV injection of NMU on Fos expression, either NMU (1.0 nmol/10 μl) or saline was injected ICV ($n = 3$) at circadian time (CT)6 (where CT0 is subjective lights on and CT12 is subjective lights off). Ninety minutes after injection, rats were perfused with 2% paraformaldehyde. For Fos staining, every one to four 40-μm-thick sections out of a series were put into 0.1 M phosphate buffer in a 6-well culture plate. The floating sections were incubated at 4 °C for 24 h with anti-c-*Fos* antiserum (Santa Cruz Biotec., Santa Cruz, CA, USA). The fluorescence staining was performed using the same method as described above.

Quantitation of mRNAs in the SCN. To examine the diurnal change in the expression of NMU and NMU receptor mRNA in the SCN, rats that had been kept under conditions of constant darkness for 3 days were sacrificed every 4 h over a 24-h period. In addition, to quantify c-fos, JunB, nerve growth factor (NGF)I-A, NGFI-B, Per1, and Per2 mRNA in the SCN after ICV injection of NMU, 3 nmol NMU was injected into rats at CT6, 90 min before collection of SCN tissues for mRNA extraction.

After the brain tissues had been frozen, the SCNs were dissected out [18]. Total RNA was extracted from the SCNs using an RNeasy Mini kit (Qiagen) and then synthesized into first-strand cDNA. The quantity of first-strand cDNA in the isolated samples was determined using either a QuantiTect SYBR Green PCR kit (Qiagen) on an ABI GeneAmp5700 with the following rat NMU primer set: 5′-TGCTGCTCGCCTGCTGT-3′ (sense), 5′-TGGTGGTAATCTTTG AGGCGAT-3′ (antisense); rat NMU-R1 primer set: 5′-CACGACT CCCATAGCCA-3′ (sense), 5′-TCACACCCTGGATCCCTGTT-3′ (antisense); and rat NMU-R2 primer set: 5′-GATGAATCCCTTGA GGCGAA-3′ (sense), 5′-ATGGCAAACACGAGGACCAA-3′ (antisense). GAPDH levels were used as an internal standard. To quantify Per1, Per2, and immediate early gene expression, SCNs were dissected out 30 min after ICV injection of NMU at CT6. The primers used were: *Period1*, 5′-GGGAGCTCAAACTTCGACTGCCACCAGAG C-3′ (sense), 5′-CAGAGCTCACCCTCCTCCAGACTCC-3′ (antisense); *Period2*, 5′-CTCCCCAAGTCCCACCAGTC-3′ (sense), 5′-CG TCCCGTGGAGCAGTTCTC-3′ (antisense); *c-fos*, 5′-CCTACTGTG TTCCTGGCAATAGTGT-3′ (sense), 5′-GCGATTTATTTCTATCT ACCGGAAAA-3′ (antisense); *JunB*, 5′-CTGGAGGACAAGGTGA AGACACT-3′ (sense), 5′-ATGACCTTCTGCTTGAGCTGC-3′ (antisense); *NGFI-A*, 5′-GCATGCGTAATTTCAGTCGTAGTG-3′ (sense), 5′-GGCAAACTTTCTCCCACAAATG-3′ (antisense); and *NGFI-B*, 5′-CTCTGATTACTATGGAAGCCCCTG-3′ (sense), 5′-AGAAGTGGCCAAATGAACCATC-3′ (antisense).

Measurement of locomotor activity. Adult male Wistar rats bearing an ICV cannula were housed in individual plastic cages ($30 \times 20 \times 12$ cm) under 12:12 h light:dark cycle (lights on at 07:00–19:00 h) for 2 weeks, and then transferred to constant darkness in an isolated chamber box equipped with infrared sensors to measure the locomotor activity rhythms [19]. All movement of an animal in the cage was converted into counts, and total activity counts were collected every 15 min by a computer. ICV pulse treatment with 0.2 or 2.0 nmol NMU was performed in darkness with the help of an infrared scope and infrared illumination. The magnitude of any phase shift was estimated by visual inspection using the best-fit connecting activity offset from the free-running rhythm before and after pulse treatments.

Results and discussion

Immunohistochemical analysis indicated that NMU is expressed in the SCN (Fig. 1A), where it was concentrated preferentially in the ventrolateral subfield, exhibiting a similar distribution to that attributed to vasoactive intestinal peptide (VIP; Fig. 1A) [20,21]. We examined the expression pattern of NMU mRNA in the SCN of rats that had been kept under conditions of constant darkness for 2–3 days. We observed an oscillation of NMU expression levels, peaking at CT4–8 (Fig. 1B). The two NMU-binding receptors (NMU-R1 and NMU-R2) were also expressed in the SCN (Figs. 1C and D). NMU-R1 mRNA levels in the SCN oscillated in a circadian manner under conditions of constant darkness, being high during the subjective day and low during the subjective night. This expression pattern was similar to that of NMU mRNA. In contrast, the level of NMU-R2 mRNA in the SCN was high at CT16–20. These results indicate that the expression of NMU-R1 and NMU-R2 mRNA is regulated differently. We next examined the activation of SCN cells by NMU. ICV injection of NMU induced the expression of Fos protein in SCN cells, whereas saline had no effect (Fig. 1E). Fos was expressed preferentially in the ventrolateral subfield rather than the dorsomedial portion. In addition, administration of NMU increased significantly the number of Fos-stained cells in several brain regions including the amygdala, PVN, dorsomedial nucleus, the third ventral ependyma, the paraventricular thalamic area, and the arcuate nucleus (data not shown), as has been reported previously in rats [22].

ICV injection of NMU into rats exhibiting a free-running rhythm under conditions of constant darkness

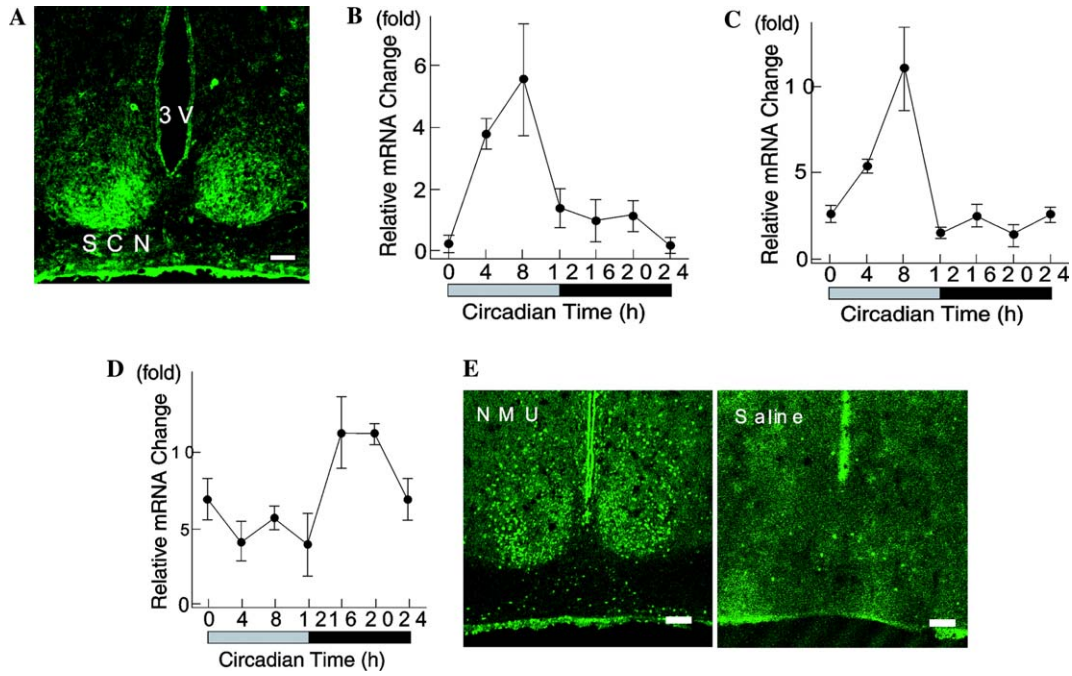

Fig. 1. NMU and the NMU receptors in the SCN. (A) Immunohistochemical detection of NMU-containing neural cells in the SCN. Scale bar: 100 μm. (B–D) NMU (B) and NMU receptor (C, NMU-R1; D, NMU-R2) mRNA levels in the SCN at different times are plotted following normalization to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Data are presented as means ± SEM ($n = 3$–5) of NMU mRNA levels. CT0 data are replotted as CT24 for clarity. (E) NMU-induced Fos protein expression in the SCN. Scale bar: 100 μm. To examine Fos expression, either NMU (1.0 nmol/10 μl) or saline was injected ICV at CT6 ($n = 3$).

induced a phase-dependent phase shift of the circadian locomotor activity rhythm, but did not influence circadian period length. Injection of NMU at CT0 delayed the rhythm, while injection at CT6 advanced it (Figs. 2A and B). Injections made at CT12 and CT18, however, had no effect (Figs. 2C and D). Fig. 2E shows the time

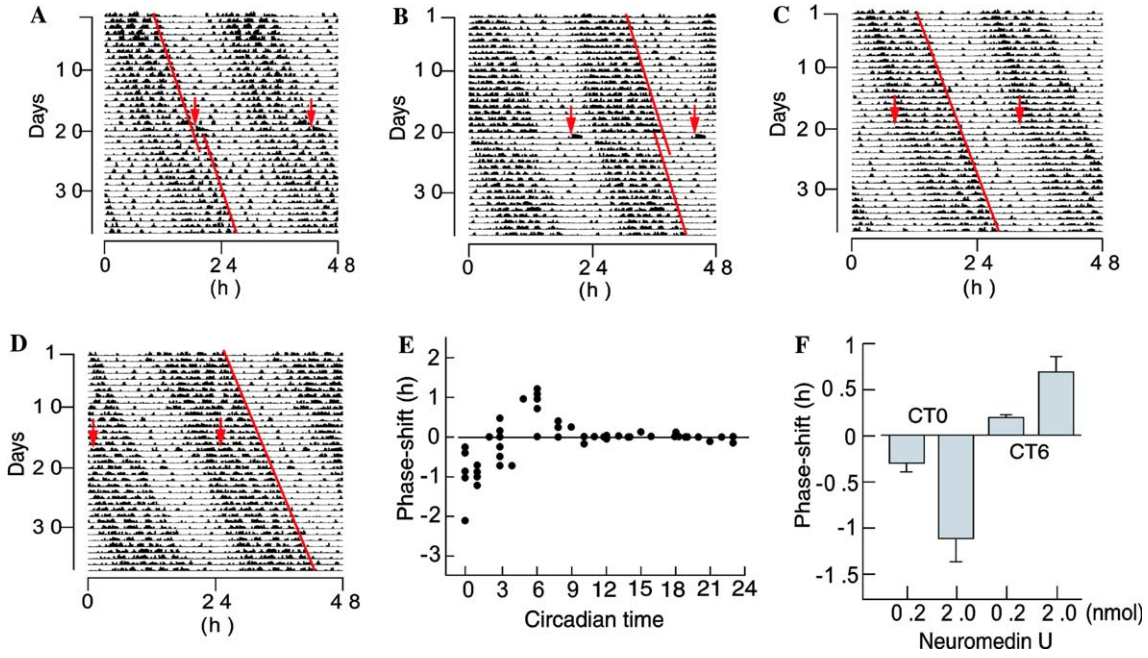

Fig. 2. NMU induced a phase shift in the circadian rhythm when administered during the subjective day. (A–D) Examples of double-plotted locomotor activity records before and after ICV injection of 2 nmol NMU at CT0 (A), CT6 (B), CT12 (C), or CT18 (D). The offset time was used to draw the line because it was more reliable than the onset time. (E) Relationship between NMU injection time and the direction or magnitude of the phase shift. (F) The temporal length of the phase shift is proportional to the dose of NMU injected.

dependency of the phase shift induced by NMU injection. NMU induced a phase shift when administered during the subjective day; thus, NMU was effective only when given during the subjective day. The phase shift induced by NMU administration was also dose dependent; the injection of higher amounts of NMU induced longer phase shifts (Fig. 2F).

We next examined mRNA expressions induced in rat SCN by ICV NMU injection (Figs. 3A–F). Quantitative RT-PCR analyses revealed increases in the expression of immediate early genes, such as *c-fos*, *NGFI-A*, *NGFI-B*, and *JunB* (Figs. 3A–D). NMU-induced Fos protein induction in the SCN was also observed with the aid of immunohistochemical analysis (Fig. 1E). These transcription factors may regulate the expression of genes involved in the circadian clock. Furthermore, ICV injection of NMU increased the expression of *Per1* (Fig. 3E), which is an essential component of the circadian clock [23,24]. The expression of *Per2* was not altered by ICV NMU injection.

In the present study, the localization of NMU and its receptors in the SCN, the cyclic 24-h expression pattern of NMU, and the NMU-induced expression of Fos in the SCN indicate that NMU is strongly related to SCN function, and if that is the case, the action of NMU is autocrine or paracrine. It is well known that several peptides, such as VIP, arginine vasopressin (AVP), somatostatin (SP), gastrin releasing peptide, and pituitary adenylate-cyclase-activating polypeptide (PACAP), are involved in the functioning of the circadian rhythm in the SCN [25–28]. Although the ventrolateral subfield localization of NMU is similar to that of VIP and PACAP, the rhythm of NMU activity in the SCN is similar to that of AVP and SP rather than PACAP and VIP [25–29]. In addition, NMU induced a phase shift of the circadian rhythm only during the subjective day, in contrast to the shift induced by light [30,31], which only induces rhythm changes during the subjective night. Nonphotic types of phase shift have been shown to be induced by peptides like NMU, for example neuropeptide Y (NPY), but NPY is not synthesized in the SCN [32]. Therefore, NMU may play a specific important role in the circadian oscillator systems. The facts that ICV injection of NMU increased the expression of immediate early genes, such as *c-fos*, *NGFI-A*, *NGFI-B*, and *JunB*, and increased the expression of *Per1* support this hypothesis [33–36]. These immediate early genes and *Per1* are also induced in the SCN by light exposure [36], although the temporal period during which light is effective differs by 12 h from the period that NMU is effective. Interestingly, the *Per1* expression induced by NMU is in the opposite direction to that observed for nonphotic stimuli such as serotonin (5-hydroxytryptamine) and a novel wheel-running activity, which suppressed *Per1* expression [37,38], although both NMU and nonphotic stimuli are effective during subjective

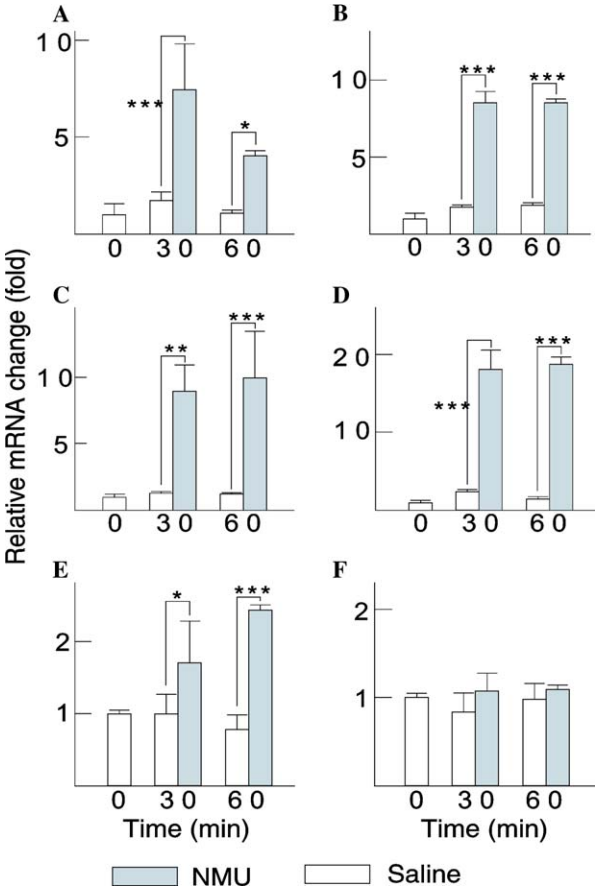

Fig. 3. ICV injection of NMU increased the mRNA levels of immediate early genes and *Per1* in the rat SCN. (A) *c-fos*, (B) *JunB*, (C) *NGFI-A*, (D) *NGFI-B*, (E) *Per1*, and (F) *Per2*. Three nanomoles of NMU was injected into rats at CT6 before collection of SCN tissues for mRNA extraction. An infusion of saline was used as a control. Quantification of mRNA levels was achieved by real-time RT-PCR. Black and white bars indicate the increases in NMU and control mRNA levels, respectively. The mRNA level at CT 0 (time 0) was assigned a value of 1. Data are presented as means ± SEM ($n = 3$). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

day. These facts suggest that NMU does not act through the typical nonphotic pathways to shift the phase of the circadian rhythm.

As the majority of oscillating proteins in the SCN contained a CACGTG E-box in their genomic promoter region [39], we searched for E-box motifs in human and mouse NMU genes. While the regulatory region of the mouse NMU gene has two CACGTG E-boxes, there is no E-box in the regulatory region of the human NMU gene. Interestingly, in the mouse genome, NMU gene maps closely to the Clock gene, which encodes a molecule that is essential for normal circadian behavior [40]. A subset of neuropeptides and receptors, including epidermal growth factor and prokineticin 2, has recently been reported to regulate circadian rhythm [41,42]. The possible autocrine or paracrine action of NMU in the SCN resembles the relationship between VIP, PACAP, and VPAC2 receptors in the SCN [43]. Further studies

are required to clarify the mechanism underlying regulation of the circadian oscillator by those proteins, including NMU. In conclusion, NMU may play some important role in regulating mammalian circadian behavior. Our results have identified NMU as a novel regulatory factor that is associated with the molecular clock.

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References

[1] N. Minamino, K. Kangawa, H. Matsuo, Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord, Biochem. Biophys. Res. Commun. 130 (1985) 1078–1085.

[2] N. Minamino, K. Kangawa, M. Honzawa, H. Matsuo, Isolation and structural determination of rat neuromedin U, Biochem. Biophys. Res. Commun. 156 (1988) 355–360.

[3] J. Domin, Y.G. Yiangou, R.A. Spokes, A. Aitken, K.B. Parmar, B.J. Chrysanthou, S.R. Bloom, The distribution, purification, and pharmacological action of an amphibian neuromedin U, J. Biol. Chem. 264 (1989) 20881–20885.

[4] J. Domin, M.A. Ghatei, P. Chohan, S.R. Bloom, Neuromedin U: a study of its distribution in the rat, Peptides 8 (1987) 779–784.

[5] S.J. Augood, J.R. Keast, P.C. Emson, Distribution and characterisation of neuromedin U-like immunoreactivity in rat brain and intestine and in guinea pig intestine, Regul. Pept. 20 (1988) 281–292.

[6] J. Ballesta, F. Carlei, A.E. Bishop, J.H. Steel, S.J. Gibson, M. Fahey, R. Hennessey, J. Domin, S.R. Bloom, J.M. Polak, Occurrence and developmental pattern of neuromedin U-immunoreactive nerves in the gastrointestinal tract and brain of the rat, Neuroscience 25 (1988) 797–816.

[7] C. Chu, Q. Jin, T. Kunitake, K. Kato, T. Nabekura, M. Nakazato, K. Kangawa, H. Kannan, Cardiovascular actions of central neuromedin U in conscious rats, Regul. Pept. 105 (2002) 29–34.

[8] D.R. Brown, F.L. Quito, Neuromedin U octapeptide alters ion transport in porcine jejunum, Eur.J. Pharmacol. 155 (1988) 159–162.

[9] A.D. Howard, R. Wang, S.S. Pong, T.N. Mellin, A. Strack, X.M. Guan, Z. Zeng, D.L. Williams, S.D. Feighner, C.N. Nunes, B. Murphy, J.N. Stair, H. Yu, Q. Jiang, M.K. Clements, C.P. Tan, K.K. McKee, D.L. Hreniuk, T.P. McDonald, K.R. Lynch, J.F. Evans, C.P. Austin, C.T. Caskey, L.H. Van der Ploeg, Q. Liu, Identification of receptors for neuromedin U and its role in feeding, Nature 406 (2000) 70–74.

[10] M. Kojima, R. Haruno, M. Nakazato, Y. Date, N. Murakami, R. Hanada, H. Matsuo, K. Kangawa, Purification and identification of neuromedin U as an endogenous ligand for an orphan receptor GPR66 (FM3), Biochem. Biophys. Res. Commun. 276 (2000) 435–438.

[11] A.M. Wren, C.J. Small, C.R. Abbott, P.H. Jethwa, A.R. Kennedy, K.G. Murphy, S.A. Stanley, A.N. Zollner, M.A. Ghatei, S.R. Bloom, Hypothalamic actions of neuromedin U, Endocrinology 143 (2002) 4227–4234.

[12] R. Hanada, M. Nakazato, N. Murakami, S. Sakihara, H. Yoshimatsu, K. Toshinai, T. Hanada, T. Suda, K. Kangawa, S. Matsukura, T. Sakata, A role for neuromedin U in stress response, Biochem. Biophys. Res. Commun. 289 (2001) 225–228.

[13] R. Raddatz, A.E. Wilson, R. Artymyshyn, J.A. Bonini, B. Borowsky, L.W. Boteju, S. Zhou, E.V. Kouranova, R. Nagorny, M.S. Guevarra, M. Dai, G.S. Lerman, P.J. Vaysse, T.A. Branchek, C. Gerald, C. Forray, N. Adham, Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system, J. Biol. Chem. 275 (2000) 32452–32459.

[14] F.K. Stephan, I. Zucker, Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions, Proc. Natl. Acad. Sci. USA 69 (1972) 1583–1586.

[15] R.Y. Moore, V.B. Eichler, Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat, Brain Res. 42 (1972) 201–206.

[16] M.U. Gillette, S.A. Tischkau, Suprachiasmatic nucleus: the brain's circadian clock, Rec. Prog. Horm. Res. 54 (1999) 33–58.

[17] T. Ida, K. Nakahara, T. Katayama, N. Murakami, M. Nakazato, Effect of lateral cerebroventricular injection of appetite stimulating neuropeptide, orexin and neuropeptide Y, on the various behavioral patterns in rats, Brain Res. 821 (1999) 526–529.

[18] M.S. Mondal, M. Nakazato, Y. Date, N. Murakami, M. Yanagisawa, S. Matsukura, Widespread distribution of orexin in rat brain and its regulation upon fasting, Biochem. Biophys. Res. Commun. 256 (1999) 495–499.

[19] N. Marumoto, N. Murakami, T. Katayama, H. Kuroda, T. Murakami, Effects of daily injections of melatonin on locomotor activity rhythms in rats maintained under constant bright or dim light, Physiol. Behav. 60 (1996) 767–773.

[20] J.P. Card, N. Brecha, H.J. Karten, R.Y. Moore, Immunocytochemical localization of vasoactive intestinal polypeptide-containing cells and processes in the suprachiasmatic nucleus of the rat: light and electron microscopic analysis, J. Neurosci. 1 (1981) 1289–1303.

[21] Y. Takahashi, H. Okamura, N. Yanaihara, S. Hamada, S. Fujita, Y. Ibata, Vasoactive intestinal peptide immunoreactive neurons in the rat suprachiasmatic nucleus demonstrate diurnal variation, Brain Res. 497 (1989) 374–377.

[22] Y. Ozaki, T. Onaka, M. Nakazato, J. Saito, K. Kanemoto, T. Matsumoto, Y. Ueta, Centrally administered neuromedin U activates neurosecretion and of c-fos messenger ribonucleic acid in the paraventricular and supraoptic nuclei of rat, Endocrinology 143 (2002) 4320–4329.

[23] B. Zheng, U. Albrecht, K. Kaasik, M. Sage, W. Lu, S. Vaishnav, Q. Li, Z.S. Sun, G. Eichele, A. Bradley, C.C. Lee, Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock, Cell 105 (2001) 683–694.

[24] K. Bae, X. Jin, E.S. Maywood, M.H. Hastings, S.M. Reppert, D.R. Weaver, Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock, Neuron 30 (2001) 525–536.

[25] C. Fukuhara, K. Shinohara, K. Tominaga, Y. Otori, S.T. Inouye, Endogenous circadian rhythmicity of somatostatin like-immunoreactivity in the rat suprachiasmatic nucleus, Brain Res. 606 (1993) 28–35.

[26] S.T. Inouye, S. Shibata, Neurochemical organization of circadian rhythm in the suprachiasmatic nucleus, Neurosci. Res. 20 (1994) 109–130.

[27] K. Shinohara, K. Tominaga, Y. Isobe, S.T. Inouye, Photic regulation of peptides located in the ventrolateral subdivision of the suprachiasmatic nucleus of the rat: daily variations of

vasoactive intestinal polypeptide, gastrin-releasing peptide, and neuropeptide Y, J. Neurosci. 13 (1993) 793–800.
[28] C. Fukuhara, N. Suzuki, Y. Matsumoto, Y. Nakayama, K. Aoki, G. Tsujimoto, S.T. Inouye, Y. Masuo, Day-night variation of pituitary adenylate cyclase-activating polypeptide (PACAP) level in the rat suprachiasmatic nucleus, Neurosci. Lett. 229 (1997) 49–52.
[29] K. Tominaga, K. Shinohara, Y. Otori, C. Fukuhara, S.T. Inouye, Circadian rhythms of vasopressin content in the suprachiasmatic nucleus of the rat, Neuroreport 3 (1992) 809–812.
[30] J.J. Milette, F.W. Turek, Circadian and photoperiodic effects of brief light pulses in male Djungarian hamsters, Biol. Reprod. 35 (1986) 327–335.
[31] J.M. Kornhauser, D.E. Nelson, K.E. Mayo, J.S. Takahashi, Photic and circadian regulation of c-fos gene expression in the hamster suprachiasmatic nucleus, Neuron 5 (1990) 127–134.
[32] K. Shinohara, K. Tominaga, C. Fukuhara, Y. Otori, S.I. Inouye, Processing of photic information within the intergeniculate leaflet of the lateral geniculate body: assessed by neuropeptide Y immunoreactivity in the suprachiasmatic nucleus of rats, Neuroscience 56 (1993) 813–822.
[33] M.E. Morris, N. Viswanathan, S. Kuhlman, F.C. Davis, C.J. Weitz, A screen for genes induced in the suprachiasmatic nucleus by light, Science 279 (1998) 1544–1547.
[34] E.L. Sutin, T.S. Kilduff, Circadian and light-induced expression of immediate early gene mRNAs in the rat suprachiasmatic nucleus, Brain Res. Mol. Brain Res. 15 (1992) 281–290.
[35] B. Rusak, H.A. Robertson, W. Wisden, S.P. Hunt, Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus, Science 248 (1990) 1237–1240.
[36] Y. Shigeyoshi, K. Taguchi, S. Yamamoto, S. Takekida, L. Yan, H. Tei, T. Moriya, S. Shibata, J.J. Loros, J.C. Dunlap, H. Okamura, Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript, Cell 91 (1997) 1043–1053.
[37] K. Horikawa, S. Yokota, K. Fuji, M. Akiyama, T. Moriya, H. Okamura, S. Shibata, Nonphotic entrainment by 5-HT1A/7 receptor agonists accompanied by reduced Per1 and Per2 mRNA levels in the suprachiasmatic nuclei, J. Neurosci. 20 (2000) 5867–5873.
[38] E.S. Maywood, N. Mrosovsky, M.D. Field, M.H. Hastings, Rapid down-regulation of mammalian period genes during behavioral resetting of the circadian clock, Proc. Natl. Acad. Sci. USA 96 (1999) 15211–15216.
[39] N. Gekakis, D. Staknis, H.B. Nguyen, F.C. Davis, L.D. Wilsbacher, D.P. King, J.S. Takahashi, C.J. Weitz, Role of the CLOCK protein in the mammalian circadian mechanism, Science 280 (1998) 1564–1569.
[40] L.D. Wilsbacher, A.M. Sangoram, M.P. Antoch, J.S. Takahashi, The mouse Clock locus: sequence and comparative analysis of 204 kb from mouse chromosome 5, Genome Res. 10 (2000) 1928–1940.
[41] A. Kramer, F.C. Yang, P. Snodgrass, X. Li, T.E. Scammell, F.C. Davis, C.J. Weitz, Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling, Science 294 (2001) 2511–2515.
[42] M.Y. Cheng, C.M. Bullock, C. Li, A.G. Lee, J.C. Bermak, J. Belluzzi, D.R. Weaver, F.M. Leslie, Q.Y. Zhou, Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus, Nature 417 (2002) 405–410.
[43] A.J. Harmar, H.M. Marston, S. Shen, C. Spratt, K.M. West, W.J. Sheward, C.F. Morrison, J.R. Dorin, H.D. Piggins, J.C. Reubi, J.S. Kelly, E.S. Maywood, M.H. Hastings, The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei, Cell 109 (2002) 497–508.