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Short communication

Fentanyl, a μ-opioid receptor agonist, phase shifts the hamster circadian pacemaker

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Abstract

The phase-shifting effects of the μ-opioid receptor agonist fentanyl on the circadian timing system were investigated in the hamster. Fentanyl injections during the mid-subjective day induced phase advances of the hamsters' wheel-running activity rhythm. The shifts were not accompanied by an increase in locomotor activity but instead a decrease of activity was often observed. A dose–response curve indicated that with increasing dosage, the response probability increased, while the magnitude of the induced shift remained stable. The present data suggest that there is some role for opioid regulation of the circadian system.

Theme: Neural basis of behaviour

Topic: Biological rhythms and sleep

Keywords: Circadian rhythm; Phase shift; Entrainment; Non-photic; Morphine; Opioid; Fentanyl; μ Receptor; Superior colliculus

A major pacemaker for circadian rhythms is located in the suprachiasmatic nuclei (SCN) at the base of the anterior hypothalamus [19,20,29]. This pacemaker drives many physiological functions [13]. Circadian rhythms are adjusted to the environmental light–dark cycle via the retina [16,41]. Light pulses applied during the beginning of night result in phase delays of the circadian cycle, whereas at the end of the night they cause phase advances [14,27,28,34]. During the day, light does not phase shift the pacemaker. The time-dependent responsiveness to light assures that the circadian pacemaker entrains to the environmental light–dark cycle.

Although light appears to be effective only at night, other stimuli appear to have phase shifting effects mainly during the day. For example, increased running wheel activity, which can be induced by novel wheel access or cage cleaning produces large phase-advances [10,30]. Moreover, several drugs and neurotransmitters such as benzodiazepines, serotonin and neuropeptide Y induce phase advances when applied during the day [1,3,6,17,22,36–38]. The function of these phase shifting responses at daytime is unknown.

The influence of morphines on the circadian system has recently received increasing attention [2,11,18]. In one study in mice, morphine was shown to induce shifts in the circadian rhythm of running wheel activity [11]. These shifts were attributable to an increase in the animals' activity following injection, and not to their direct effect on SCN neurons. In a pilot study we investigated the effects of fentanyl, a potent μ-opioid receptor agonist on the activity rhythm of hamsters, and found no increase in activity levels following fentanyl application. This offers the possibility to investigate the effects of this opioid on the circadian pacemaker in isolation from activating effects and to specifically address the question whether activation

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of the μ-opioid receptor affects the circadian pacemaker's phase.

Male golden hamsters (*Mesocricetus auratus*) were obtained from Harlan, Nederland, Horst, The Netherlands, at the age of 10 weeks (80–100 g). The animals were housed in a sound attenuated and temperature-controlled room, in individual cages that were equipped with running wheels. Wheel-running activity was recorded and stored automatically by a computer system, with a time-resolution of 1 min. Hamsters were entrained to a light–dark regimen (L:D=14:10) with a light intensity of about 100 lux at the bottom of the cages, before they were released in constant darkness.

Phase response curve: After 7 days in darkness, animals received intraperitoneal fentanyl injections (0.1 mg, 2 ml) aimed at one of the circadian times 3, 6, 9, 12, 15, 18, 21 and 24, with circadian time 12 defined as the onset of running wheel activity. After the injection, the animals were kept in constant darkness for another 2 weeks. Phase shifts were determined by fitting straight lines through activity onsets before and after the injection. The fitted lines were extrapolated to the day immediately following the injection to measure phase advances (+) or delays (−). The animals were re-entrained to a light dark cycle and the whole procedure was repeated with a lower dose of fentanyl (0.025 mg) and finally with control injections (NaCl, 2 ml). Each animal contributed at most two times to each phase response plot. When animals received more than one injection within the same time epoch, mean values were taken. The fentanyl-induced shifts that were obtained at the various circadian times were compared with phase shifts induced by control injections and differences were tested with an ANOVA test.

Dose response plot: To determine the dose–response relationship for fentanyl, animals additionally received injections of 0.05 mg fentanyl aimed at CT 6. The experimental protocol was similar to that described above. Data were pooled between CT 4.5 and 7.5. To quantify the percentage of animals that responded with a phase shift, we used a criteria based on our control experiments. The highest phase shift obtained with control injection (0.5 h, $N=1$) was considered to be a nonspecific response. Phase shifts above this value were considered to be meaningful fentanyl-induced shifts. The percentage of animals that responded with phase shifts >0.5 h were plotted as a function of the dosage with the 95% confidence intervals included. Of those animals that responded with a phase shift, the average phase shift (±2 S.E.M.) was calculated.

A total number of 51 animals received fentanyl or control injections at various circadian phases. Fentanyl injections that were centered around CT 6 and CT 9 induced phase advances of the circadian activity rhythm (Fig. 1). At CT 6, a mean phase shift of 1.3 h was observed (confidence interval±0.31) and at CT 9 it was 0.8 h±0.18. At other circadian times, large phase advances or delays were absent. A reduction in running wheel activity was often observed for about one circadian cycle irrespective of the timing of the injection (Fig. 1A and B). In only two instances, fentanyl injections were accompanied by an increase in running wheel activity.

A phase response curve (PRC) was obtained for both 0.1 mg and 0.025 mg fentanyl injections (Fig. 2). The fentanyl induced phase shifts differed significantly between circadian times ($P<0.001$) for the highest dosage of fentanyl. In particular, at CT 6 and CT 9 the average phase shift was significantly higher than at CT 12 ($P<0.001$, $P=0.038$, respectively). The PRC for fentanyl differs moreover from the PRC for control injections ($P<0.001$, Fig. 2). In the control group there were no differences between the circadian times ($P=0.26$).

At around CT 6, we separately analyzed the effects of control injections ($N=6$ animals) and injections with 0.025 mg fentanyl ($N=13$), 0.5 mg fentanyl ($N=17$) and 0.1 mg fentanyl ($N=19$). For control injections, no significant phase shifts were observed. For the respective dosages of 0.025 mg, 0.05 mg and 0.1 mg fentanyl, we observed a response probability of 15%, 59% and 90% respectively (Fig. 3A). These data could be fitted by a sigmoid curve, described by:

$$y = \{\exp(-2.6986 + 2.6517x)\} / \{1 + \exp(-2.6986 + 2.6517x)\}$$

with x = dosage and y = response probability.

We additionally analyzed the magnitudes of the fentanyl induced phase shifts irrespective of the dosage, and we observed a bimodal distribution; animals responded either with no significant phase change, or they responded with a shift (Fig. 3B). The non-responsive group displayed an average phase change of 0.11 h (S.D.: 0.013) whereas the other group responded with a mean shift of 1.30 h (S.D.: 0.33). Hardly any phase shifts were observed between 0.4 and 0.8 h. The data could be fitted significantly better with two curves instead of one curve ($X^2=23.6$, $df=3$, $P<0.0001$). The point where the curves intersect coincides with the value that we applied to discriminate between responsive and non-responsive animals and justifies the criteria that we applied.

Of those animals that responded with a phase shift >0.5 h, mean phase shifts were determined as a function of dosage (Fig. 3C). Mean phase shifts (±2 S.E.M.) for dosages 0.1, 0.05 and 0.025 mg, were 1.0 h (±0.14, $N=2$), 1.15 h (±0.29, $N=10$), and 1.48 h (±0.26, $N=17$). These shifts were not significantly different.

The present data indicate that fentanyl induces phase advances when injected during the subjective day, and no phase shifts when applied during the subjective night. The shifts during the day differed significantly from shifts induced by control injections and we conclude therefore that they are attributable to the action of fentanyl itself. A complete reduction of activity was often observed in the first hours following the injection and the animals usually

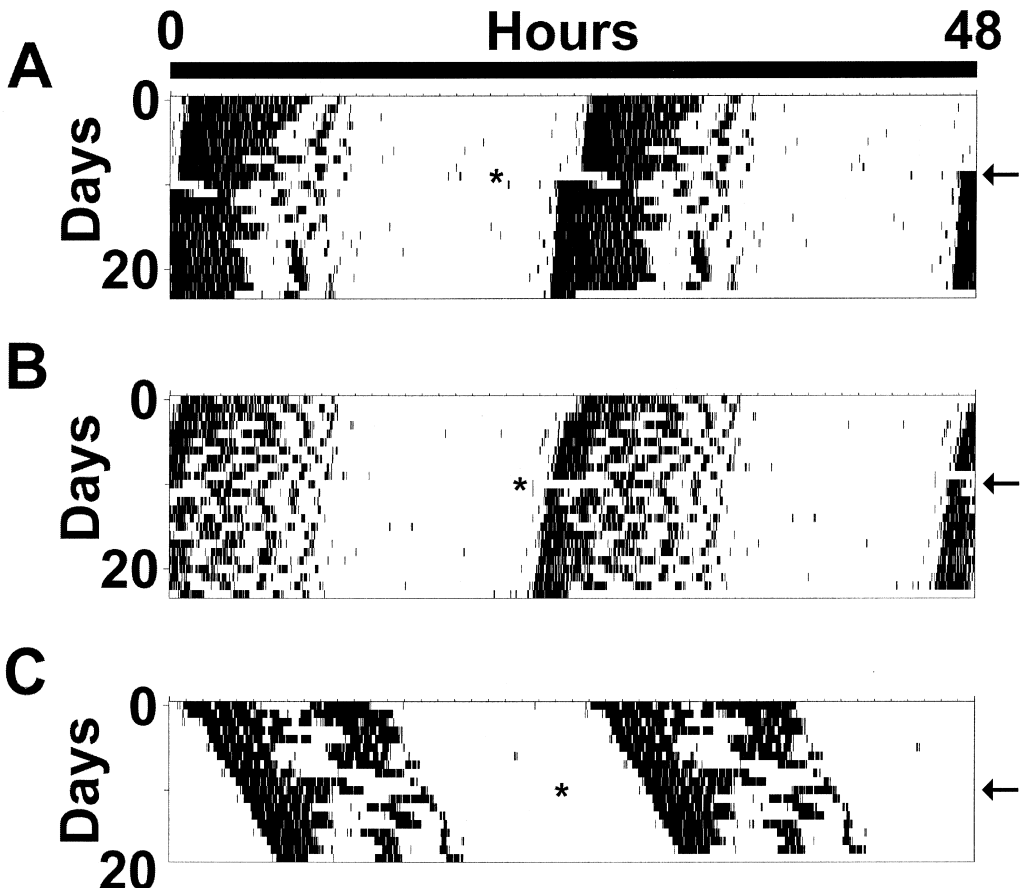

Fig. 1. The phase shifting effect of fentanyl on the circadian activity rhythm of a hamster. (A) Double plotted running wheel activity rhythm of the hamster. After an initial entrainment period the hamster was released in constant darkness. After 7 days the animal received a fentanyl injection at circadian time 7. The day of the injection is indicated by an arrow and the timing by an asterisk. The injection induced a decrease in running wheel activity and a phase advance of the freerunning activity rhythm. (B) The animal received a fentanyl injection at CT 10.4, at the day indicated by an arrow. Although the injection induced a clear suppression in running wheel activity, it did not induce a significant phase shift. (C) The animal received a control injection (NaCl, 2 ml) at CT 7 at the indicated day. The injection did not induce a shift in the activity rhythm.

fell asleep. The question arises whether this reduction in activity induced the shift. However, lower dosages of fentanyl given at CT 6 or 9 often produced similar decreases in activity but these were not accompanied by a phase shift. Moreover, 0.1 mg injections at circadian times other than CT 6 and 9 were also accompanied by decreased activity, but rarely by phase shifts. We, therefore, argue that the decreased activity levels had no relation with the occurrence of phase shifts.

Phase shifts were maximal at CT 6, and somewhat smaller at CT 9. Although some individual delays were observed at around CT 24, mean shifts at around this time were not significant. The phase response curve for fentanyl resembles the phase response curves for a number of other phase shifting manipulations. In vivo application of neuropeptide Y, serotonin, and benzodiazepines as well as electrical stimulation of the intergeniculate leaflet result in phase advancing shifts during the day in most (but not all) studies [1,6,17,22,32,36–38]. Increased locomotor activity induced by cage cleaning and novel wheel access result in similar advances during the day [10,30,37]. Differences between the curves exist with respect to the time where optimal advances are obtained (CT 6–CT 9) and with respect to the presence of a phase delaying area in the PRC. Fentanyl has a clear optimum at CT 6 and shows no significant phase delays.

A dose response curve was obtained for injections that were performed between CT 4.5 and CT 7.5. A peculiar finding of this study was that an increment in dosage resulted in an increase in response probability, but not so much in an increase in the magnitude of the response. When phase shifts were plotted against the frequency of their occurence (Fig. 3B) a bimodal distribution emerged. This was interpreted as an area of nonresponders and an area with responders. Almost no phase shifts were induced with a magnitude between 0.4 and 0.8 h for any of the used dosages.

These findings suggest that there is not a gradual change in responsiveness to fentanyl, but that instead the transition in responsiveness is very abrupt. Animals either respond within a certain range of values, or they do not respond to the drug. It is possible that this particular dose response

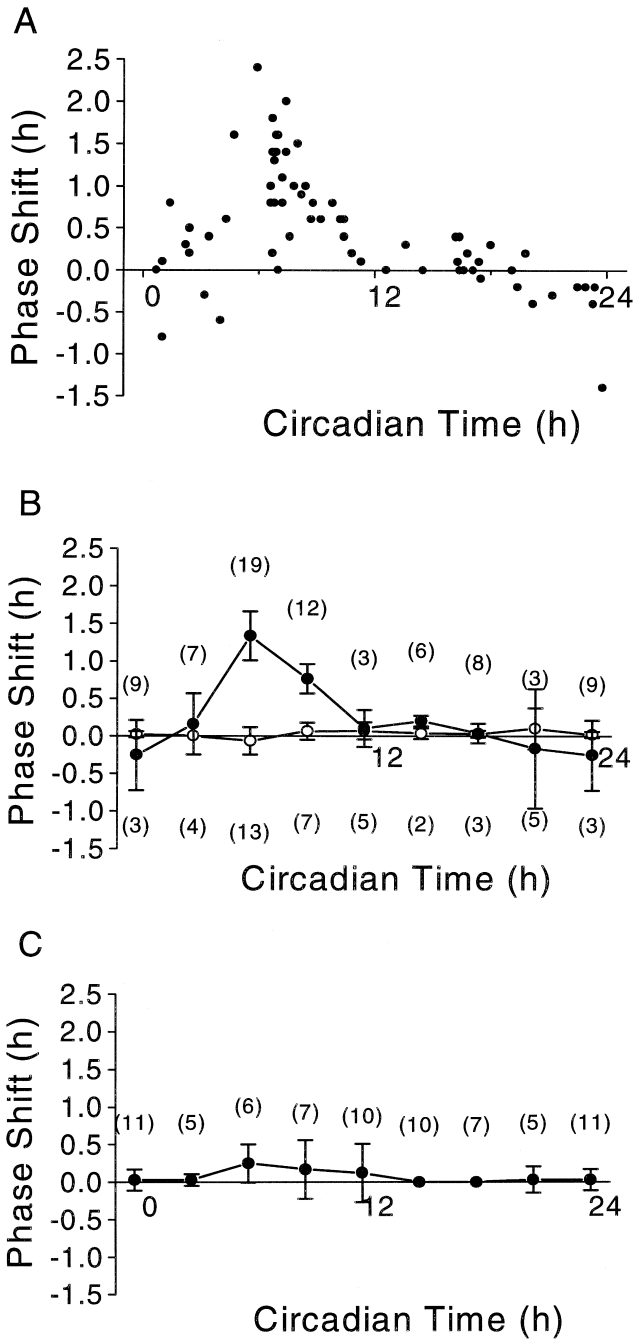

Fig. 2. Phase response plots for the effects of fentanyl (0.1 and 0.025 mg) and control injections. (A) Individual phase shifts induced by fentanyl (0.1 mg) are plotted against the circadian time of the injection. Circadian time 12 corresponds with the onset of running wheel activity. (B) The mean shifts induced by 0.1 mg fentanyl injections (closed symbols) and control injections (open symbols). Number of used animals is indicated between brackets above the error bars (experimental animals) or below the error bars (control animals). Circadian times refer to the midpoints of the injection times and data were pooled between CT 1.5–CT 4.5, between CT 4.5–CT 7.5 and so on. (C) Phase response plot for the phase shifting effect of 0.025 mg fentanyl.

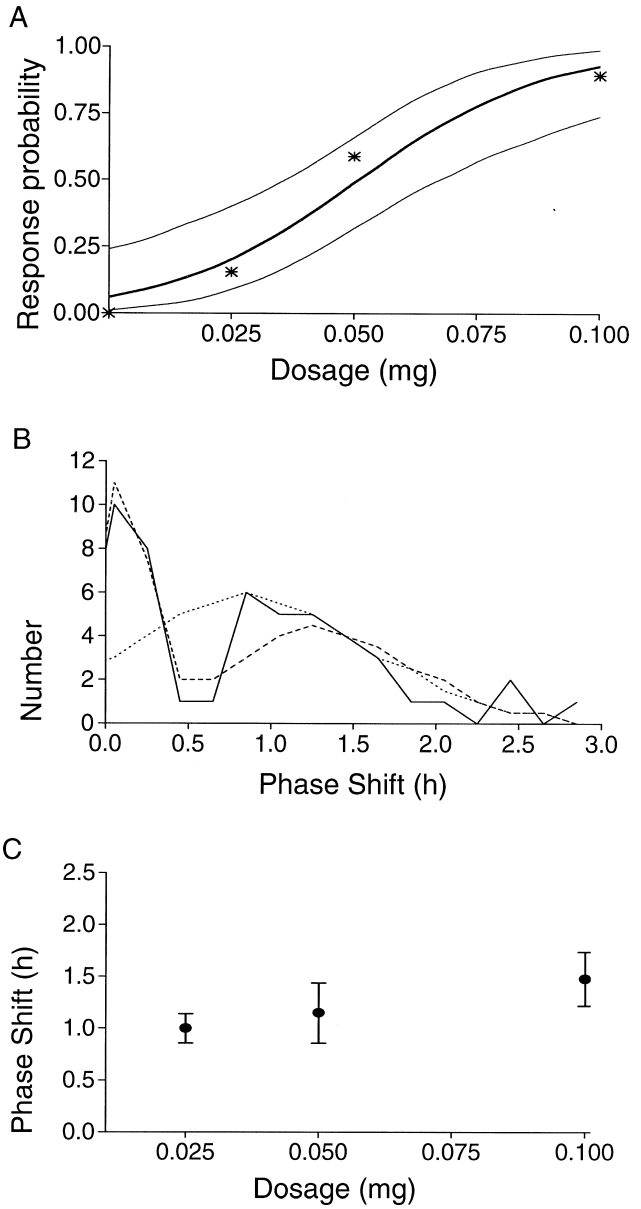

Fig. 3. Dose–response effects of fentanyl at mid-subjective day. (A) Increased dosages of fentanyl induce an increase in response probability. The data have been fitted by a sigmoid curve and 95% confidence intervals have been indicated. (B) The number of phase shifts of a certain magnitude have been plotted against the magnitude of the phase shifts (solid lines). These data could be fitted significantly better by a bimodal distribution (dashed line) than by a unimodal distribution (dotted line). (C) For those animals that responded with a phase shift the magnitude of the phase shifts was plotted as a function of dosage. The mean response did differ not for the used dosages of fentanyl.

relationship indicates that a certain cascade of events is triggered by a minimal dosage, and that the effect is of the all-or-none type. Alternatively, there may be a very small working area. For instance, the intensity response curve for light effects on the pacemaker is characterized by a very small working area, especially when it is compared to the light intensities that actually occur in the environment [14,15,34]. Similar properties may exist for the responsiveness to μ-opioid receptor stimulation.

In the recent years fruitful research has been directed to identify afferent pathways involved in day-time phase

shifting [17]. In short, a role for both the intergeniculate leaflet and the raphe nucleus is indicated. However, we know only little of the underlying mechanism by which phase shifts are induced at daytime. As most drugs induce an increase in running wheel activity, the general notion has emerged that it is not the drug itself but rather its specific side effects that trigger phase shifts during the day. This idea was underscored by the finding that increased activity by itself has phase shifting effects at daytime, and that interference with behavioral activity prevents the drug-induced phase shifts. However, the benzodiazepine chlordiazepoxide induces phase shifts without concomitant change in activity [3]. Our present findings indicate that phase shifting by fentanyl occur without increment in behavioral activity.

The absence of activity induction in response to fentanyl stands in contrast to the activating effect of morphine in mice [11]. The lack of activation in our hamsters may be somewhat surprising because in the rat, the activating effect of morphine appears to be mediated by the μ-receptor [8]. Possibly, species differences as well as different activation patterns of the various μ-receptor subtypes by morphine and fentanyl may be responsible for the differences in activation.

If the phase shifting effect of fentanyl is not indirectly mediated by increased activity levels of the animal, the question arises where fentanyl has exerted its action? There are tree possibilities and that is (1) in the SCN itself via a newly identified OFQ/N receptor subtype, (2) in the SCN itself, via yet unidentified receptors and (3) by activation of afferent pathways that are involved in non-photic phase shifting.

Recent in situ hybridization studies have revealed expression of orphanin-FQ/nociceptin (OFQ/N receptor (NOR) mRNA in the SCN [2]. A large majority of SCN neurons respond in a dose-dependent way to OFQ/N with an outward current. Fentanyl shows weak affinity for the OFQ/N receptor subtype. However, local application of OFQ/N into the SCN of hamsters failed to induce phase shifts of the behavioral activity rhythms of these animals. Therefore, it is very unlikely that fentanyl induced shifts were mediated by the OFQ/N receptor.

The geniculohypothalamic tract (GHT) projects to the SCN with fibres containing enkephalines [20,21,24–26,35]. The role of these enkephalines for the circadian system has received little attention. Enkaphlines act on three classes of opioid receptors, that is on μ, delta (δ) and kappa (κ) receptors through activation of G-protein coupled receptors [31,33]. It is possible that fentanyl injections have exerted their effect through activation of opioid receptors in the SCN [5]. However, Cutler et al. (1999) [4] have demonstrated in vitro that SCN neurons were generally unresponsive to the opioid receptor agonists leucine–enkephalin, methionine–enkephalin or morphine. Taken together, this option deserves further study.

The superior colliculus has recently been implicated as one of the brain areas that mediate phase shifting to triazolam [12]. The superior colliculus shows reciprocal connections with the intergeniculate leaflet [9,23,25] and projects also directly to the SCN in the rat, cat and rabbit [7,39,40]. Stimulation of the μ-receptor in the superior colliculus may therefore have influenced the activity of SCN neurones indirectly via activation of the intergeniculate leaflet.

The present data show that stimulation of the μ-opioid receptor by fentanyl has phase shifting effects and it is suggested that endogenous opioids may play a significant role in the circadian timing system. Possible phase shifting effects of exogenous administered opioids in patients should be considered.

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References

[1] H.E. Albers, C.F. Ferris, Neuropeptide Y: Role in light-dark entrainment of hamster circadian rhythms, Neurosci. Lett. 50 (1984) 163–168.
[2] C.N. Allen, Z.-G. Jiang, K. Teshima, T. Darland, M. Ikeda, C.S. Nelson, D.I. Quigley, T. Yoshioka, R.G. Allen, M.A. Rea, D.K. Grandy, Orphanin-FQ/Nociceptin (OFQN) modulates the activity of suprachiasmatic nucleus neurons, J. Neurosci. 19 (1999) 2152–2160.
[3] S.M. Biello, N. Mrosovski, Circadian phase-shifts induced by chlordiazepoxide without increased locomotor activity, Brain Res. 622 (1993) 58–62.
[4] D.J. Cutler, M.K. Mundey, R. Mason, Electrophysiological effects of opioid receptor activation on Syrian hamster suprachiasmatic nucleus neurones in vitro, Brain Res. Bull. 50 (1999) 119–125.
[5] G.C. Desjardins, J.R. Brawer, A. Beaudet, Distribution of mu, delta and kappa opioid receptors in the ventromedial hypothalamus of the rat, Brain Res. 536 (1990) 114–123.
[6] D.M. Edgar, J.D. Miller, R.A. Prosser, R.R. Dean, W.C. Dement, Serotonnin and the mammalian circadian system. II. Phase-shifting rat behavioral rhythms with serotonergic agonists, J. Biol. Rhythms 8 (1993) 17–31.
[7] I. Fabian, A. Ableitner, Brain sites involved in mu-opioid receptor-mediated actions: a 2-deoxyglucose study, Brain Res. 697 (1995) 205–215.
[8] M. Grung, S. Skurtveit, T.A. Aasmundstad, M. Handal, R.L. Alkana, J. Morland, Morphine-6-glucuronide-induced locomotor stimulation in mice:role of opioid receptors, Pharmacol. Toxicol 1 (1998) 3–10.
[9] M.E. Harrington, The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated strictures in the visual and circadian systems, Neurosci. Biobehav. Rev. 21 (1997) 705–727.
[10] D. Janik, N. Mrosovski, Nonphotically induced phase shifts of circadian rhythms in the golden hamster: Activity–response curves at different ambient temperatures, Physiol. Behav. 53 (1993) 431–436.
[11] E.G. Marchant, R.E. Mistlberger, Morphine Phase-shifts circadian

rhythms in mice: role of behavioural activation, Neuroreport 7 (1995) 209–212.
[12] E.G. Marchant, L.P. Morin, The hamster circadian rhythm system includes nuclei of the subcortical visual shell, J. Neurosci. 19 (1999) 10482–10493.
[13] J.H. Meijer, W.J. Rietveld, Neurophysiology of the suprachiasmatic circadian pacemaker in rodents, Physiol. Rev. 69 (1989) 671–707.
[14] J.H. Meijer, B. Rusak, G. Gänshirt, The relation between light-induced phase shifts in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms, Brain Res. 598 (1992) 257–263.
[15] J.H. Meijer, K. Watanabe, J. Schaap, H. Albus, L. Détári, Light responsiveness of the suprachiasmatic nucleus: long-term multiunit and single-unit recordings in freely moving rats, J. Neurosci. 18 (1998) 9078–9087.
[16] J.H. Meijer, Functional absence of extraocular photoreception in hamster circadian rhythm entrainment, Brain Res. 831 (1999) 337–339.
[17] E.L. Meyer-Bernstein, L.P. Morin, Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation, J. Neurosci. 16 (1996) 2097–2111.
[18] R.E. Mistlberger, M.M. Holmes, Morphine-induced activity attenuates phase shifts to light in C57BL/6J mice, Brain Res. 829 (1999) 113–119.
[19] R.Y. Moore, V.B. Eichler, Loss of adrenal corticosterone rhythm following suprachiasmatic nucleus lesions in the rat, Brain Res. 42 (1972) 201–206.
[20] R.Y. Moore, J.C. Speh, GABA is the principle nerotransmitter of the circadian system, Neurosci. Lett. 150 (1993) 112–116.
[21] R.Y. Moore, J.P. Card, Intergeniculate leaflet; An anatomically and functionally distinct subdivision of the lateral geniculate complex, J. Comp. Neurol. 344 (1994) 403–439.
[22] L.P. Morin, Neural control of circadian rhythms as revealed through the use of benzodiazepines, in: D.C. Klein, R.Y. Moore, S.M. Reppert (Eds.), Suprachiasmatic Nucleus: The Mind's Clock, Oxford University Press, New York, 1991, pp. 324–338.
[23] L.P. Morin, The circadian visual system, Brain Res. Rev. 67 (1994) 102–127.
[24] L.P. Morin, J. Blanchard, R.Y. Moore, Intergeniculate leaflet and suprachiasmatic nucleus organization and connections in the golden hamster, Vis. Neurosci. 8 (1992) 219–230.
[25] L.P. Morin, J. Blanchard, Organization of the hamster intergeniculate leaflet and posterior limitans nucleus, Vis. Neurosci. 12 (1995) 57–67.
[26] L.P. Morin, J. Blanchard, Interconnections among nuclei of the subcortical visual Shell: the intergeniculate leaflet is a major constituent of the hamster subcortical visual system, J. Comp. Neurol. 6 (1998) 288–309.
[27] D.E. Nelson, J.S. Takahashi, Sensitivity and integration in a visual pathway for circadian entraiment in the hamster (Mesocricetus auratus), J. Physiol. 439 (1991) 115–145.
[28] C.S. Pittendrigh, S. Daan, A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock, J. Comp. Physiol. 106 (1976) 291–331.
[29] M.R. Ralph, R.G. Foster, F.C. Davis, M. Menaker, Transplanted suprachiasmatic nucleus determines circadian period, Science 247 (1990) 975–978.
[30] S.G. Reebs, N. Mrosovski, Effects of induced wheel running on the circadian activity rhythms of Syrian hamsters: Entrainment and PRC, J. Biol. Rhythms 4 (1989) 39–48.
[31] T. Reisine, Opiate receptors, Neuropharmacology 34 (1995) 463–472.
[32] B. Rusak, J.H. Meijer, M.E. Harrington, Hamster circadian rhythms are phase-shifted by electrical stimulation of the geniculo-hypothalamic tract, Brain Res. 493 (1989) 283–291.
[33] M. Satoh, M. Minami, Molecular pharmacology of the opioid receptors, Pharmacol. Ther. 68 (1995) 343–364.
[34] J.S. Takahashi, P.J. DeCoursey, L. Bauman, M. Menaker, Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms, Nature 308 (1984) 186–188.
[35] K. Takatsuji, M. Tohyama, Geniculo-geniculate projection of enkephalin and Neuropeptide Y containing neurons in the intergeniculate leaflet of the thalamus in the rat, J. Chem. Neuroanat. 2 (1989) 19–27.
[36] K. Tominaga, S. Shibata, S. Ueki, S. Watanabe, Effects of 5-HT_{1A}receptor agonists on the circadian rhythm of wheel-running activity in hamsters, Eur. J. Pharmacol. 214 (1992) 79–84.
[37] F.W. Turek, Effects of stimulated physical activity on the circadian pacemaker of vertebrates, J. Biol. Rhythms 4 (1989) 135–147.
[38] O. Van Reeth, F.W. Turek, Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines, Nature 339 (1989) 49–51.
[39] J.M. Walker, W.D. Bowen, L.A. Thompson, J. Frascella, S. Lehmkuhle, H.C. Hughes, Distribution of opiate receptors within visual structures of the cat brain, Exp. Brain Res. 73 (1988) 523–532.
[40] T. Wichmann, R.B. Illing, K. Startke, Evidence for a neurotransmitter function of acetylcholine in rabbit superior colliculus, Neuroscience 23 (1987) 991–1000.
[41] S. Yamazaki, M. Goto, M. Menaker, No evidence for extraocular photoreceptions in the circadian system of the Syrian hamster, J. Biol. Rhythms 14 (1999) 197–201.