
LOOKING AHEAD

Drugs of abuse may have differential effects on the expression of clock genes and the modulation of clock genes' expression may interfere with the effect of drugs of abuse.

The Effects of Drugs of Abuse on Clock Genes

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The interrelationship between drugs of abuse and biological rhythms has been observed for many years. While the endogenous nature of the daily rhythm of life was revealed in humans in the early 1960s, clinical reports did not uncover the existence of a circadian rhythm in the effects of drugs such as methadone or alcohol until a decade later.¹⁻⁵ In the 1970s a clear 24-hour periodicity of presentations of addicted patients was shown in emergency departments, peaking in the early evening.^{6,7} Furthermore, several animal studies confirmed the daily variations of the effects of a drug. For instance, in rats amphetamine has been shown to affect differently motor responses depending on the time of the day, and a clear 24-hour cycle in the analgesic effect of morphine has been demonstrated.^{8,9}

In the meantime, our knowledge of the daily rhythms of life has evolved tremendously. The daily fluctuation of the body state is under the control of

Summary

Daily fluctuations of the behavioral and pharmacological effects of drugs of abuse such as cocaine, morphine or alcohol are observed for several years. Since the discovery of the molecular components of the biological clock, the so-called "clock genes", several studies have further confirmed the inter-relationship between drugs of abuse and biological rhythms. Indeed, drugs of abuse have been shown to clearly induce specific expression changes in clock genes depending on the brain area, the mode of administration or the specific clock gene. On the other hand, increasing evidence for a clear involvement of several clock genes in the development of several drug-induced behaviors has been shown. The present review summarizes these recent findings and reveals the complexity, as well as the specificity, of the interrelation between drugs of abuse and clock genes. © 2008 Prous Science, S.A.U. or its licensors. All rights reserved.

an endogenous biological clock, the so-called "master clock", which is located in the suprachiasmatic nucleus of the hypothalamus, and synchronized by environmental stimuli such as the light-dark cycle or food availability. The master clock orchestrates our endocrine, physiological and behavioral functions by synchronizing peripheral clocks located in other brain areas (i.e., striatum, prefrontal cortex) or in peripheral organs (i.e., liver and kidney) using neuronal and endocrine pathways.¹⁰⁻¹² The molecular clockwork of the master and peripheral clocks has been identified and conceptualized (Fig. 1). Several "clock" genes (i.e., *Per*, *Clock*, *Bmal1*, *Cry* genes) interact with each other in regulatory transcriptional and transla-

tional feedback loops thereby self-sustaining a circadian period of activity of the cell and modulating the expression of clock-controlled genes.¹³⁻¹⁵ Different clock genes (i.e., *Per1* and *Per2* genes) may affect different clock-controlled genes and thereby modulate the expression of diverse functions in different brain areas or peripheral tissues.

It is suggested that (i) drugs of abuse may have differential effects on the expression of clock genes and that (ii) the modulation of clock genes' expression may interfere with the effect of drugs of abuse. In the present review, we will summarize the recent

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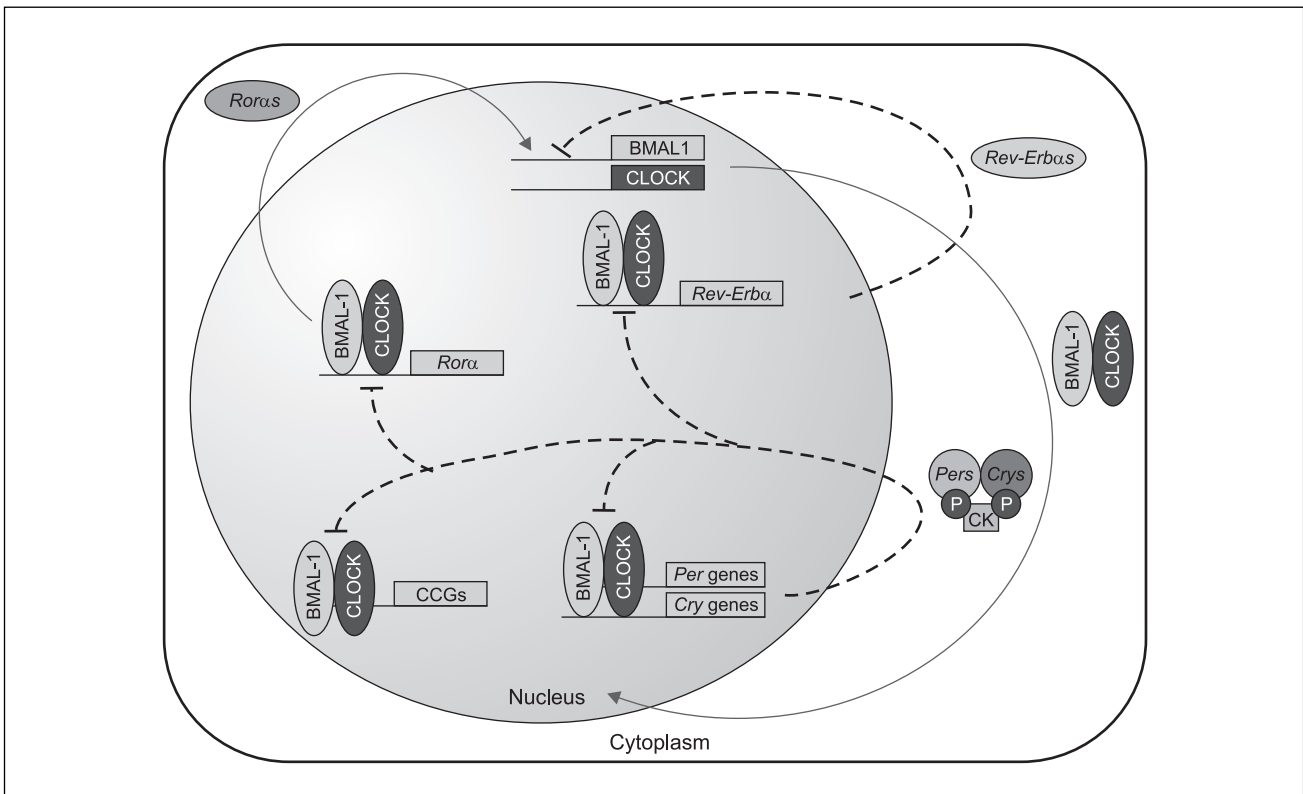


Fig. 1. Molecular clockwork. This model consists of interactive positive (straight gray arrows) and negative (dashed black arrows) feed-back molecular loops. The BMAL-1 and the CLOCK proteins form heterodimers that activate upon their entry into the cell nucleus the transcription of several genes (i.e., *Per1-3*, *Cry1-2*, *Rev-Erb α* , *Rora*) through their E-box enhancers. Once translated, these proteins regulate the activity of the complex CLOCK/BMAL-1 within the nucleus. The different *Per* and *Cry* genes can also modulate the expression of the clock-controlled genes (CCGs), thereby modulating the rhythmic output of the cell.

findings confirming the importance of clock genes in terms of the action of several drugs of abuse, and we will propose putative neurobiological explanations for such functions.

Psychostimulants and clock genes

Several animal studies have revealed that psychostimulants differentially affect the expression of clock genes in various brain regions. Masubuchi et al.¹⁶ have shown, for instance, that chronic methamphetamine treatment in daytime desynchronizes locomotor activity in rats. In addition, the authors showed that methamphetamine treatment has no effect on the rhythmic expression of *rPer1* gene within the suprachiasmatic nucleus, but desynchronizes and even reverses the rhythm of expression of *rPer1*, *rPer2* and *rBmal1* genes within the striatum or the parietal cortex. This first observation has been

further confirmed by other studies in mice. Nikaido et al.¹⁷ have shown that repeated injections of methamphetamine cause a sensitized increase in *mPer1* gene expression specifically in the mouse striatum without affecting *mPer2* or *mPer1* gene expression within the master clock. In addition, Iijima et al.¹⁸ have demonstrated that an acute injection of methamphetamine increases the expression of the *mPer1*, *mBmal1* and *mNpas2* genes in the striatum, and that a chronic daytime methamphetamine treatment shifts the rhythmic *mPer1* and *mPer2* expression in the striatum from a nocturnal to a diurnal rhythm, but fails to affect that in the suprachiasmatic nucleus. Furthermore, binge administration of cocaine also leads to a long-lasting upregulation of *rPer2* expression in the rat frontal cortex and the striatum, as shown in a microarray analysis.¹⁹ Interestingly, Uz et al.²⁰ have shown that in mice cocaine

affects the expression of clock genes differently depending on the treatment schedule (acute or chronic) and depending on the brain area (hippocampus, striatum, prefrontal cortex). For example, they showed an upregulation of the *mClock* gene specifically in the striatum but not in the hippocampus after both acute and chronic cocaine administration. The *mPer1* gene is upregulated in both regions after chronic treatment, while the *mPer2* gene is only upregulated in the hippocampus following both treatment schedules and is downregulated in the striatum following chronic cocaine administration. Furthermore, Shang and Zhdanova²¹ have recently shown that a single prenatal exposure to cocaine can dysregulate in a dose-dependent manner the expression of the zebra fish clock genes *zBmal1* and *zPer3*, and that these effects are stronger during the daytime. Altogether, these results confirm the com-

plexity of the action of psychostimulants on clock gene expression in terms of the specific clock gene, brain area or the time of the day.

In addition to the direct effects of psychostimulants on the expression of clock genes, several studies have revealed the importance of clock genes in drug-induced behaviors such as sensitization and conditioned place preference, both behavioral phenomena that are known to be involved in the development of addictive behavior.^{22,23} In the late 1990s, Andretic et al.²⁴ made a tremendous finding using a new model of repeated volatilized free-base cocaine administration in *Drosophila* flies. In this study the authors showed that *Drosophila* flies mutant for period, clock, cycle and double-time genes were not able to express behavioral sensitization following repeated administration of cocaine. A few years later, after the generation of *Per1^{Brdm1}* and *Per2^{Brdm1}* mutant mice, these findings could be replicated in mice and could be extended to the modulation of cocaine reinforcement processes.^{25,26} Thus, it was demonstrated that both behavioral sensitization and conditioned place preference induced by cocaine are absent in *Per1^{Brdm1}* mutant mice, whereas these phenomena are expressed even more strongly in *Per2^{Brdm1}* mutant mice.²⁷ In addition, this study showed that these two behavioral phenomena involved in the development of cocaine addiction showed diurnal differences, with a higher degree of sensitization and reinforcement at the beginning of the light phase (start of the resting period of the animals). Since this first study, the role of the *Per1* gene in the development of cocaine sensitization has been confirmed in successive studies conducted in different rodents.^{28–31} These studies showed the involvement of endogenous melatonin in driving the rhythm in PER1 protein expression within the striatum, which, as the authors suggested, would be responsible for the rhythm in cocaine-induced sensitization. Meanwhile, McClung et al.³² have also shown the involvement

of the clock gene *mClock* in modulating the cocaine-induced reinforcement, by showing that *mClock* mutant mice display an increased conditioned place preference following cocaine treatment.

When taking all these results together, one can assume that different clock genes may affect different neurobiological systems involved in drugs of abuse. As mentioned above, melatonin has been proposed to be a serious candidate for driving the rhythmicity of the *Per1* gene in the areas involved in drug dependence. On the other hand, our knowledge of the upstream systems modulated by clock genes is still not well defined. Several neurotransmitter systems are involved in the development of cocaine addiction. In particular the midbrain dopamine system and the glutamatergic system play a critical role in cocaine-induced sensitization and reinforcement.^{33–35} Both systems are influenced by the biological clock. Thus, a clear circadian rhythm in dopamine and glutamate release has been measured in the rat nucleus accumbens, peaking during nighttime.³⁶ In addition, since the dopamine D2 receptor responsiveness is under circadian control and dependent on the normal function of the *Drosophila period* gene, one may expect a particular interaction between some clock genes and the dopamine system.³⁷ Recently, this idea was further confirmed by McClung et al.³² who showed a clear relationship between the clock gene *Clock*, cocaine-induced reinforcement and dopaminergic transmission. Thus, *mClock* mutant mice showed an increase in dopamine cell firing and bursting in the ventral tegmental area while exhibiting increased sensitization and conditioned place preference. On the other hand, *mPer* genes might also be linked with *N*-methyl-D-aspartate (NMDA) receptor function, as pretreatment with an NMDA receptor antagonist prevented methamphetamine-induced increase in *mPer1* mRNA in the caudate putamen.¹⁷ Since several genes involved in these two pathways pos-

sess putative E-box domains in their promoter, the current opinion is that these genes are modulated by clock genes, therefore they can be considered direct or indirect clock-controlled genes.

Alcohol and clock genes

Rodents are nocturnal animals and exhibit a diurnal activity pattern, being more active during nighttime or the dark phase. This circadian behavior consequentially affects other behavioral variables and thus it follows that nocturnal animals will consume significantly more food and fluids during their active phase.³⁸ Similarly, this diurnal rhythmicity is also observed with respect to alcohol consumption and preference, both being clearly more elevated in the dark phase.^{38–40} Circadian rhythmicity has also been observed with regard to ethanol-induced hypothermia and the hypnotic effects of this drug on the central nervous system.^{41–44} Why is it that certain effects of alcohol differ according to the time of the day? One logical explanation for this circadian phenomenon may point to a possible temporal variation in ethanol metabolism and consequentially varying blood alcohol levels and elimination rates. Over the last few decades, only a limited number of studies have tackled the issue of ethanol metabolism and circadian rhythmicity, arriving at very conflicting conclusions. Early, questionable studies in humans claimed to have seen such a temporal effect.^{45–47} However, later studies could not confirm this assertion, as was demonstrated by Yap et al.⁴⁸ and Lötterle et al.,⁴⁹ who could not observe a circadian elimination rate of ethanol. Moreover, similar negative results were obtained in rodents, with Sato et al.⁴⁴ also excluding a temporal variation in hepatic metabolism.⁴²

Conversely to the diurnal rhythmicity of alcohol consumption, alcohol administration can also influence biological rhythms (e.g., body temperature, glucocorticoids, etc.) in animals and humans; alcohol has especially

pronounced disruptive effects on sleep, demonstrating a reciprocal relation between biological rhythms and alcohol.^{50–54}

In line with the observations that alcohol can influence biological rhythms, two studies have now clearly shown on the molecular level that alcohol affects the expression of clock genes in different brain areas. Thus, Chen et al.⁵⁵ have shown that chronic ethanol administration induces long-lasting upregulation of *rPer2* expression in the rat frontal cortex and striatum. In addition, the same group has demonstrated that prenatal ethanol exposure alters the expression of clock genes in the arcuate nucleus and the suprachiasmatic nucleus of the hypothalamus.⁵⁶

Per1^{Brdm1} and *Per2^{Brdm1}* mutant mice have now also been studied in alcohol self-administration experiments. Using operant conditions, *Per1^{Brdm1}* and wild-type mice were trained to self-administer alcohol. Furthermore, extinction sessions were introduced, followed by reinstatement measures of ethanol-seeking behavior. In another set of animals, the mice were exposed to voluntary long-term alcohol consumption, followed by a 2-month deprivation phase, after which the alcohol deprivation effect, which is used as a measure of relapse, was examined. Mutant mice did not display a significantly divergent number of reinforced lever presses than wild-type animals. Furthermore, no significant differences between groups were obtained regarding reinstatement of ethanol-seeking behavior. Similar results were obtained in the two-bottle free choice paradigm. Specifically, no genotype differences concerning consumption and preference were observed over a broad range of different ethanol concentrations. Moreover, after the deprivation phase, both groups exhibited significant alcohol deprivation effects, yet no genotype differences.⁵⁷ These data do not suggest a relationship between the circadian clock gene *mPer1* and ethanol reinforcement, ethanol-seeking and

relapse behavior. In contrast, mutant mice compared to wild-type animals exhibit an enhanced alcohol intake and preference when pharmacologically relevant concentrations of 8–16% ethanol are offered in a two-bottle free choice test.⁵⁸ It was further demonstrated that the caloric value, taste differences and variations in alcohol elimination cannot account for the enhanced alcohol intake in these mice.⁵⁸ Alterations in the brain reinforcement system of these mutant mice might therefore drive an enhanced incentive motivation to consume more alcohol than control animals do. The mesolimbic reinforcement system is modulated by various glutamatergic input pathways. In a series of experiments it was found that *Per2^{Brdm1}* mutant mice have a hyperglutamatergic state, especially in the nucleus accumbens.⁵⁸ Regarding the large body of evidence given in the literature for an involvement of enhanced glutamate levels or alterations of the glutamatergic system in excessive alcohol consumption, one would expect a massive impact of the *Per2* gene mutation on alcohol consumption via alterations within the glutamatergic system.^{59–64} This idea has been further confirmed by examining the effects of acamprosate in these mice. Acamprosate is used in the clinic for relapse prevention, and it is suggested that acamprosate acts mainly on a hyperglutamatergic state while having only little effect on a “normal” glutamatergic state.^{65,66} Therefore, acamprosate should be more effective in reducing alcohol consumption in *Per2^{Brdm1}* mutant than in wild-type mice. Indeed, following repeated acamprosate treatment, mutant mice showed decreased alcohol consumption along with a normalization of extracellular glutamate levels in the nucleus accumbens.

These new findings provide a clear link of the mouse *Per2* gene, the glutamatergic system and excessive alcohol consumption. However, future animal research ought to address the question of whether the *Per2* gene and other clock genes are also directly

implicated in alcohol sensitivity, tolerance, withdrawal and in alcohol relapse behavior. Most importantly, however, the link between *Per2* and excessive alcohol consumption in mice was already translated to humans. Thus, association studies in different samples have demonstrated that specific genetic variations of the human *PER2* gene are associated with high alcohol consumption.^{58,67}

Opioids and clock genes

When one looks at the existing literature, long reporting a clear interaction of biological rhythms and opiate abuse from the first clinical reports to the most recent animal studies, an important role of *Per* genes in modulating the effect of opiates should be expected. In 1967, Morris and Lutsch⁶⁸ already revealed a daily rhythm in morphine-induced analgesia, and clinical reports showed daily variations in methadone-induced mortality.^{2,4} Evidence for a daily rhythm in opiate analgesia has been confirmed in mice, and daily variations in susceptibility to the morphine-induced hyperactivity have been shown in rats.^{9,69–71} Important in this context is the finding of binding studies that a daily rhythm in opioid receptors does occur.⁷²

Also, evidence exists for an effect of opiate abuse on diverse biological rhythms. For instance, opiate-dependent patients suffer severely from sleep disturbances.⁷³ In addition, a relationship between biological rhythms and morphine withdrawal has long been known since the disruption of locomotor activity rhythm is used to identify spontaneous morphine withdrawal in rodents.^{74,75} Also, morphine withdrawal is associated with enhanced activity in neurons of the suprachiasmatic nucleus, and a higher degree of tolerance is observed when morphine is administered at night.^{9,76}

Recently, a gene expression profiling study has revealed that the *Per2* gene is specifically overexpressed in the prefrontal cortex following chronic morphine treatment and naloxone-

precipitated withdrawal, a finding which suggests an important role of this specific gene in morphine-induced withdrawal processes.^{77,78} Opioids also affect the expression of clock genes in different parts of the brain. Indeed, Wang et al.⁷⁹ have shown that chronic morphine exposure shifts the mouse mPER1 immunoreactivity in the brain and blunts its rhythm in the liver. The same group has further revealed that mPER1 is necessary for the expression of conditioned place preference induced by morphine.⁸⁰ Furthermore, the authors suggested that the action of *mPer1* is mediated by the activation of the extracellular signal-regulated kinase pathway, since morphine-induced extracellular signal-regulated kinase activity is downregulated following application of the *mPer1* DNA enzyme.⁸¹

The involvement of *Per1* and *Per2* in the development of tolerance to the analgesic effects of morphine was studied by the tail-immersion and hot-plate tests in *Per1^{Brdm1}* and *Per2^{Brdm1}* mutant mice. *Per2^{Brdm1}* mutant mice showed a significant decrease in their degree of tolerance to morphine when compared to wild-type littermates. In contrast, this effect could not be observed in *Per1^{Brdm1}* mutant animals. Also, withdrawal signs, measured after withdrawal precipitation with naloxone, were not significantly different in *Per1^{Brdm1}* mutant mice from those observed in control mice. On the contrary, withdrawal signs were attenuated in *Per2^{Brdm1}* mutant mice compared to their wild-type littermates.⁸² These results show that the mouse *Per2* gene is involved in the development of tolerance to the analgesic effect of morphine as well as in the expression of morphine withdrawal responses. However, the *Per1* gene does not seem to be involved in opiate tolerance and withdrawal. These opposing results demonstrate the need for further studies in order to understand the neurobiological mechanisms underlying the action of clock genes in opioid-induced behaviors.

Conclusions

In the present review, clear evidence for an inter-relationship between drugs of abuse, circadian rhythmicity and clock genes has been provided. Psychoactive drugs have the potential to influence the expression of clock genes. As a consequence, certain circadian functions may become either transiently or permanently altered. The latter effect may even result in a pathological condition, resembling many aspects of drug addiction.⁸³ Conversely, recent studies in mutated *Drosophila* flies and mouse models have revealed that the activity of clock genes influences the efficacy of drugs of abuse, findings that are also supported by human genetic studies (⁵⁸, but see also ⁸⁴). One may assume that these effects of clock genes on drugs of abuse are due to their differential interaction with several neurotransmitter systems (via the modulation of clock-controlled genes) known to be involved in the development of addictive behaviors. In fact, it has been shown that dopaminergic signaling is influenced by the *Per1* and *clock* gene whereas the activity of the *Per2* gene modulates the glutamatergic system. Furthermore, it is suggested that the effects of clock genes on the efficacy of drugs of abuse is independent of the suprachiasmatic nucleus and other brain sites that might be involved in this phenomenon. The use of *in vivo* inducible and reversible tetracycline-controlled transactivator system associated with specific clock genes, and/or the use of tissue-specific rescue of mutant mice will certainly be of a great help in identifying the brain sites involved in the interplay of drugs of abuse and clock genes.^{85,86}

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References

- 1 Aschoff, J. and Wever, R. *Spontanperiodik des menschen bei ausschluss aller zeitgeber*. *Naturwissenschaften* 1962, 49: 337–42.
- 2 Lenox, R.H. and Frazier, T.W. *Methadone induced mortality as a function of the circadian cycle*. *Nature* 1972, 239(5372): 397–8.

- 3 Sinnett, E.R. and Morris, J.B. *Temporal patterns of the use of non-prescribed drugs*. *Percept Mot Skills* 1977, 45(3 Pt. 2): 1239–45.
- 4 Argyle, E. *Circadian variation of the lethality of methadone*. *Nature* 1973, 242(5396): 332.
- 5 Reinberg, A. et al. *Variations circadiennes des effets de l'ethanol et de l'ethanolamie chez l'homme adulte sain. Etude chronopharmacologique*. *J Physiol* 1975, 70: 435–56.
- 6 Raymond, R.C., Warren, M., Morris, R.W. and Leikin, J.B. *Periodicity of presentations of drugs of abuse and overdose in an emergency department*. *J Toxicol Clin Toxicol* 1992, 30(3): 467–78.
- 7 Erickson, T.B., Lee, J., Zautcke, J.L. and Morris, R. *Analysis of cocaine chronotoxicology in an urban ED*. *Am J Emerg Med* 1998, 16(6): 568–71.
- 8 Gaytan, O., Swann, A. and Dafny, N. *Diurnal differences in rat's motor response to amphetamine*. *Eur J Pharmacol* 1998, 345(2): 119–28.
- 9 Yoshida, M., Ohdo, S., Takane, H., Tomiyoshi, Y., Matsuo, A., Yukawa, E. and Higuchi, S. *Chronopharmacology of analgesic effect and its tolerance induced by morphine in mice*. *J Pharmacol Exp Ther* 2003, 305(3): 1200–5.
- 10 Perreau-Lenz, S., Pévet, P., Buijs, R.M. and Kalsbeek, A. *The biological clock: the bodyguard of temporal homeostasis*. *Chronobiol Int* 2004, 21(1): 1–25.
- 11 Yoo, S.H., Yamzaki, S., Lowrey, P.L. et al. *PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues*. *Proc Natl Acad Sci U S A* 2004, 101(15): 5339–46.
- 12 Gachon, F., Nagoshi, E., Browns, S.A., Ripperker, J. and Schibler, U. *The mammalian circadian timing system: from gene expression to physiology*. *Chromosoma* 2004, 113(3): 103–12.
- 13 Albrecht, U. and Eichele, G. *The mammalian circadian clock*. *Curr Opin Genet Dev* 2003, 13(3): 271–7.
- 14 Ko, C.H. and Takahashi, J.S. *Molecular components of the mammalian circadian clock*. *Hum Mol Genet* 2006, 15 Spec No 2, R271–7.
- 15 Delaunay, F. and Laudet, V. *Circadian clock and microarrays: mammalian genome gets rhythm*. *Trends Genet* 2002, 18(12): 595–7.
- 16 Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M. and Honma, K. *Clock genes outside the suprachiasmatic nucleus involved in manifestation of locomotor activity rhythm in rats*. *Eur J Neurosci* 2000, 12(12): 4206–14.
- 17 Nikaido, T., Akiyama, M., Moriya, T. and Shibata, S. *Sensitized increase of period gene expression in the mouse caudate/putamen caused by repeated injection of*

- methamphetamine*. *Mol Pharmacol* 2001, 59(4): 894–900.
18. Iijima, M., Nikaido, T., Akiyama, M., Moriya, T. and Shibata, S. *Methamphetamine-induced, suprachiasmatic nucleus-independent circadian rhythms of activity and mPer gene expression in the striatum of the mouse*. *Eur J Neurosci* 2002, 16(5): 921–9.
 19. Yuferov, V., Krosiak, T., Laforqe, K.S., Zhou, Y., Ho, A. and Kreek, M.J. *Differential gene expression in the rat caudate putamen after "binge" cocaine administration: advantage of triplicate microarray analysis*. *Synapse* 2003, 48(4): 157–69.
 20. Uz, T., Ahmed, R., Akhisaroglu, M., Kurtuncu, M., Imbesi, M., Dirim Arslan, A. and Manev, H. *Effect of fluoxetine and cocaine on the expression of clock genes in the mouse hippocampus and striatum*. *Neuroscience* 2005, 134(4): 1309–16.
 21. Shang, E.H. and Zhdanova, I.V. *The circadian system is a target and modulator of prenatal cocaine effects*. *PLoS ONE* 2007, 2, e587.
 22. Sanchis-Segura, C. and Spanagel, R. *Behavioural assessment of drug reinforcement and addictive features in rodents: An overview*. *Addict Biol* 11:2–38.
 23. Tzschentke, T. *Measuring reward with the conditioned place preference (conditioned place preference) paradigm: Update of the last decade*. *Addict Biol* 12: 227–462.
 24. Andretic, R., Chaney, S. and Hirsch, J. *Requirement of circadian genes for cocaine sensitization in Drosophila*. *Science* 1999, 285(5430): 1066–8.
 25. Zheng, B., Albrecht, U., Kaasik, K. et al. *Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock*. *Cell* 2001, 105(5): 683–94.
 26. Zheng, B., Larkin, D.W., Albrecht, U. et al. *The mPer2 gene encodes a functional component of the mammalian circadian clock*. *Nature* 1999, 400(6740): 169–73.
 27. Abarca, C., Albrecht, U. and Spanagel, R. *Cocaine sensitization and reward are under the influence of circadian genes and rhythm*. *Proc Natl Acad Sci U S A* 2002, 99(13): 9026–30.
 28. Uz, T., Javaid, J.I. and Manev, H. *Circadian differences in behavioral sensitization to cocaine: putative role of arylalkylamine N-acetyltransferase*. *Life Sci* 2002, 70(25): 3069–75.
 29. Uz, T., Akhisaroglu, M., Ahmed, R. and Manev, H. *The pineal gland is critical for circadian period1 expression in the striatum and for circadian cocaine sensitization in mice*. *Neuropsychopharmacology* 2003, 28(12): 2117–23.
 30. Kurtuncu, M., Arslan, A.D., Akhisaroglu, M., Manev, H. and Uz, T. *Involvement of the pineal gland in diurnal cocaine reward in mice*. *Eur J Pharmacol* 2004, 489(3): 203–5.
 31. Akhisaroglu, M., Ahmed, R., Kurtuncu, M., Manev, H. and Uz, T. *Diurnal rhythms in cocaine sensitization and in Period1 levels are common across rodent species*. *Pharmacol Biochem Behav* 2004, 79(1): 37–42.
 32. McClung, C.A., Sidiropoulou, K., Vitaterna, M., Takahashi, J.S., White, F.J., Cooper, D.C. and Nestler, E.J. *Regulation of dopaminergic transmission and cocaine reward by the Clock gene*. *Proc Natl Acad Sci U S A* 2005, 102(26): 9377–81.
 33. Spanagel, R. and Weiss, F. *The dopamine hypothesis of reward: past and current status*. *Trends Neurosci* 1999, 22(11): 521–7.
 34. Vanderschuren, L.J. and Kalivas, P.W. *Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies*. *Psychopharmacology (Berl)* 2000, 151(2-3): 99–120.
 35. Cornish, J.L. and Kalivas, P.W. *Cocaine sensitization and craving: differing roles for dopamine and glutamate in the nucleus accumbens*. *J Addict Dis* 2001, 20(3): 43–54.
 36. Castañeda, T.R., de Prado, B.M., Prieto, D. and Mora, F. *Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light*. *J Pineal Res* 2004, 36(3): 177–85.
 37. Andretic, R. and Hirsh, J. *Circadian modulation of dopamine receptor responsiveness in Drosophila melanogaster*. *Proc Natl Acad Sci U S A* 2000, 97(4): 1873–8.
 38. Hiller-Sturmhofel, S. and Kulkosky, P. *Chronobiological regulation of alcohol intake*. *Alcohol Res Health* 2001, 25(2): 141–8.
 39. Freund, G. *Alcohol consumption and its circadian distribution in mice*. *J Nutr* 1970, 100(1): 30–6.
 40. Jelic, P., Shih, M.F. and Taberner, P.V. *Diurnal variation in plasma ethanol levels of TO and CBA mice on chronic ethanol drinking or ethanol liquid diet schedules*. *Psychopharmacology (Berl)* 1998, 138(2), 143–50.
 41. Lagerspetz, K.Y. *Diurnal variation in the effects of alcohol and in the brain 5-hydroxytryptamine metabolism in mice*. *Acta Pharmacol Toxicol (Copenh)* 1972, 31: 509–20.
 42. Deimling, M.J. and Schnell, R.C. *Circadian rhythms in the biological response and disposition of ethanol in the mouse*. *J Pharmacol Exp Ther* 1980, 213(1): 1–8.
 43. Baird, T.J., Briscoe, R.J., Vallett, M., Vanecek, S.A., Holloway, F.A. and Gauvin, D.V. *Phase-response curve for ethanol: alterations in circadian rhythms of temperature and activity in rats*. *Pharmacol Biochem Behav* 1998, 61(3): 303–15.
 44. Sato, Y., Seo, N. and Kobahashi, E. *The dosing-time dependent effects of intravenous hypnotics in mice*. *Anesth Analg* 2005, 101(6): 1706–8.
 45. Jones, B.M. and Paredes, A. *Circadian variation of ethanol metabolism in alcoholics*. *Br J Addict Alcohol Other Drugs* 1974, 69(1): 3–10.
 46. Sturtevant, F.M. *Chronopharmacokinetics of ethanol. I. Review of the literature and theoretical considerations*. *Chronobiologia* 1976, 3(3): 237–62.
 47. Minors, D.S. and Waterhouse, J.M. *Aspects of chronopharmacokinetics and chronergy of ethanol in healthy man*. *Chronobiologia* 1980, 7(4): 465–80.
 48. Yap, M., Mascord, D.J., Starmer, J.A. and Whitfield, J.B. *Studies on the chronopharmacology of ethanol*. *Alcohol Alcohol* 1993, 28(1): 17–24.
 49. Lötterle, J., Husslein, E.M., Bolt, J. and Wirtz, P.M. *Diurnal differences in alcohol absorption*. *Blutalkohol* 1998, 26(6): 369–75.
 50. Danel, T. and Touitou, Y. *Chronobiology of alcohol: From chronokinetics to alcohol-related alterations of the circadian system*. *Chronobiol Int* 2004, 21(6): 923–35.
 51. Kakihana, R. and Moore, J.A. *Circadian rhythm of corticosterone in mice: the effect of chronic consumption of alcohol*. *Psychopharmacologia* 1976, 46(3): 301–5.
 52. Rosenwasser, A.M. *Alcohol, antidepressants, and circadian rhythms. Human and animal models*. *Alcohol Res Health* 2001, 25(2): 126–35.
 53. Danel, T., Libersa, C. and Touitou, Y. *The effect of alcohol consumption on the circadian control of human core body temperature is time dependent*. *Am J Physiol Regul Integr Comp Physiol* 2001, 281(1): R52–5.
 54. Roehrs, T. and Roth, T. *Sleep, sleepiness, and alcohol use*. *Alcohol Res Health* 2001, 25(2): 101–9.
 55. Chen, C.P., Kuhn, P., Advis, J.P. and Sarkar, D.K. *Chronic ethanol consumption impairs the circadian rhythm of pro-opiomelanocortin and period genes mRNA expression in the hypothalamus of the male rat*. *J Neurochem* 2004, 88(6): 1547–54.
 56. Chen, C.P., Kuhn, P., Advis, J.P. and Sarkar, D.K. *Prenatal ethanol exposure alters the expression of period genes governing the circadian function of beta-endorphin neurons in the hypothalamus*. *J Neurochem* 2006, 97(4): 1026–33.
 57. Zghoul, T., Abarca, C., Sanchis-Segura, C., Albrecht, U., Schumann, G. and Spanagel, R. *Ethanol self-administration and reinstatement of ethanol-seeking behavior in Per1(Brdm1) mutant mice*. *Psychopharmacology (Berl)* 2007, 190(1): 13–9.
 58. Spanagel, R., Pendyala, G., Abarca, C. et al. *The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption*. *Nat Med* 2005, 11(1): 35–42.

59. Tsai, G. and Coyle, J.T. *The role of glutamatergic neurotransmission in the pathophysiology of alcoholism*. *Annu Rev Med* 1998, 49: 173–84.
60. Pulvirenti, L. and Diana, M. *Drug dependence as a disorder of neural plasticity: focus on dopamine and glutamate*. *Rev Neurosci* 2001, 12(2): 141–58.
61. Siggins, G.R., Martin G., Roberto, M. and Nie, Z., Madamba, S., De Lcea, L. *Glutamatergic transmission in opiate and alcohol dependence*. *Ann N Y Acad Sci* 2003, 1003, 196–211.
62. Heinz, A. et al. *Neurobiological correlates of the disposition and maintenance of alcoholism*. *Pharmacopsychiatry* 2003, 36 (Suppl 3): S255–258
63. Gass, J.T. and Olive, M.F. *Glutamatergic substrates of drug addiction and alcoholism*. *Biochem Pharmacol* 2008, 75(1): 218–65.
64. Krystal, J.H., Petrakis, I.L., Mason, G., Trevisan, L. and D'Souza, D.C. *N-methyl-D-aspartate glutamate receptors and alcoholism: reward, dependence, treatment, and vulnerability*. *Pharmacol Ther* 2003, 99(1): 79–94.
65. Spanagel, R. and Zieglansberger, W. *Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes*. *Trends Pharmacol Sci* 1997, 18(2): 54–9.
66. Dahchour, A. and De Witte, P. *Effects of acamprosate on excitatory amino acids during multiple ethanol withdrawal periods*. *Alcohol Clin Exp Res* 2003, 27(3): 465–70.
67. Schumann, G. *Okey Lecture 2006: identifying the neurobiological mechanisms of addictive behavior*. *Addiction* 2007, 102(11): 1689–95.
68. Morris, R.W. and Lutsch, E.F. *Susceptibility to morphine-induced analgesia in mice*. *Nature* 1967, 216(5114): 494–5.
69. Oliverio, A. et al. *Opiate analgesia: evidence for circadian rhythms in mice*. *Brain Res* 1982, 249(2): 265–70.
70. Bornschein, R.L., Crockett, R.S., and Smith, R.P. *Diurnal variations in the analgesic effectiveness of morphine in mice*. *Pharmacol Biochem Behav* 1977, 6(6): 621–6.
71. Ayhan, I.H. *Daily susceptibility variations to the morphine-induced hyperactivity of rats*. *J Pharm Pharmacol* 1974, 26(1): 76–8.
72. Naber, D., Wirz-Justice, A. and Kafka, M.S. *Circadian rhythm in rat brain opiate receptor*. *Neurosci Lett* 1981, 21(1): 45–50.
73. Oyefeso, A., Sedqwick, P. and Ghodse, H. *Subjective sleep-wake parameters in treatment-seeking opiate addicts*. *Drug Alcohol Depend* 1997, 48(1): 9–16.
74. Stinus, L., Robert, C., Karasinski, P. and Limoge, A. *Continuous quantitative monitoring of spontaneous opiate withdrawal: locomotor activity and sleep disorders*. *Pharmacol Biochem Behav* 1998, 59(1): 83–9.
75. Caillé, S., Stinus, L., Espejo, E.F., De Deurwaerdere, P., Spinato, U. and Koob, G.F. *Inhibition of 5-HT neurotransmission increases clonidine protective effects on naloxone-induced conditioned place aversion in morphine-dependent rats*. *Neuropsychopharmacology* 2003, 28(2): 276–83.
76. Cutler, D.J., Munday, M.K. and Mason, R. *Electrophysiological effects of opioid receptor activation on Syrian hamster suprachiasmatic nucleus neurones in vitro*. *Brain Res Bull* 1999, 50(2): 119–25.
77. Ammon, S., Mayer, P., Riechert, U., Tischmeyer, H. and Höllt, V. *Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal*. *Brain Res Mol Brain Res* 2003, 112(1-2): 113–25.
78. Ammon-Treiber, S. and Höllt, V. *Morphine-induced changes of gene expression in the brain*. *Addict Biol* 2005, 10(1): 81–89
79. Wang, X.W., Xin, Y., Liu, H. et al. *Altered expression of circadian clock gene, mPer1, in mouse brain and kidney under morphine dependence and withdrawal*. *J Circadian Rhythms* 2006, 4: 9.
80. Liu, Y., Wang Y., Wan, C. et al. *The role of mPer1 in morphine dependence in mice*. *Neuroscience* 2005, 130(2): 383–8.
81. Liu, Y., Wang, Y., Ziang, Z., Wan, C., Zhou, W. and Wang, Z. *The extracellular signal-regulated kinase signaling pathway is involved in the modulation of morphine-induced reward by mPer1*. *Neuroscience* 2007, 146(1): 265–71.
82. Perreau-Lenz, S. et al. *Differential involvement of mPer1 and mPer2 genes in the development of morphine-induced tolerance and withdrawal*. *Behav Pharmacol* 2007, 18(Suppl. 1): S80.
83. Perreau-Lenz, S. et al. *Clock genes running amok. Clock genes and their role in drug addiction and depression*. *EMBO Rep* 2007, 8: 20–4.
84. Malison, R.T., Kranzler, R.H., Yang, B.Z. and Galernter, J. *Human clock, PER1 and PER2 polymorphisms: lack of association with cocaine dependence susceptibility and cocaine-induced paranoia*. *Psychiatr Genet* 2006, 16(6): 245–9.
85. Hong, H.K., Chong, J.L., Song, W. et al. *Inducible and reversible Clock gene expression in brain using the tTA system for the study of circadian behavior*. *PLoS Genet* 2007, 3(2): e33.
86. McDearmon, E.L., Patel, K.L., Ko, C.H. et al. *Dissecting the functions of the mammalian clock protein BMAL1 by tissue-specific rescue in mice*. *Science* 2006, 314(5803): 1304–8.

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