



Impact of Circadian Disruption on CART mRNA Expression in Nucleus accumbens: Insights from Constant Light Exposure and Wheel-Running Activity in Rats

Fahimeh Mohseni^{1,2}, Afsaneh Vahedifar³, Shima Mohammadi^{2,4}, Kia Garmabi⁵, Atefeh Bakhtazad^{6,7}, Behzad Garmabi^{2,4*}

¹ Department of Addiction, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.

² Neuroscience Research Center, Shahroud University of Medical Sciences, Shahroud, Iran.

³ Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.

⁴ Department of Neuroscience, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.

⁵ Department of Biomedical Engineering, Faculty of Medicine, Kermanshah University of Medical Sciences (KUMS), Kermanshah, Iran.

⁶ Cellular and Molecular Research Center, Deputy of Research and Technology, Iran University of Medical Sciences, Tehran, Iran.

⁷ Department of Neuroscience, School of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.

Received: 13 April 2025

Accepted: 4 August 2025

Abstract

Background: Cocaine Amphetamine Related Transcript (CART) is expressed in the nucleus accumbens (NAc), a region that serves as extra-SCN circadian oscillators. This study examined whether CART mRNA in the NAc follows a diurnal rhythm and how circadian disruption affects its expression.

Methods: Rats (n=28) were monitored for 30 days in a 12:12 light-dark cycle (LD). On day 30, 14 rats were sacrificed (7 in the morning, 7 in the evening). The remaining rats (n=14) were exposed to constant light (LL) from day 30 to day 60 and then sacrificed (7 in the morning, 7 in the evening). CART mRNA levels were measured via real-time PCR.

Results: In the LD group, CART mRNA was higher in the evening than in the morning (P-value<0.001). In the LL group, evening levels remained elevated but were reduced compared to LD (P-value<0.01). Morning mRNA levels in the LL group were lower than in LD (P-value<0.05).

Conclusions: Constant light exposure downregulated CART mRNA, suggesting impaired circadian regulation in the NAc. This disruption may alter neurochemical signaling, affecting behavioral and mood-related processes. The findings highlight CART's role in circadian coordination and its susceptibility to rhythm disturbances, which could influence mood and activity patterns.

Keywords: Neuropeptide CART, Circadian rhythm, Nucleus accumbens, Wheel running.

*Corresponding to: B Garmabi, Email: behzad.garmabi@gmail.com

Please cite this paper as: Mohseni F, Vahedifar A, Mohammadi S, Garmabi K, Bakhtazad A, Garmabi B. Impact of Circadian Disruption on CART mRNA Expression in the Nucleus accumbens: Insights from Constant Light Exposure and Wheel-Running Activity in Rats. Shahroud Journal of Medical Sciences 2025;11(3):8-16.

Introduction

The hypothalamus of ovine was originally used by Spiess et al. in 1981 to isolate and sequence the Cocaine Amphetamine Related Transcript (CART) which was initially named somatostatin-like peptide¹. About 14 years later, however, it was renamed CART once Douglass et al. realized that this peptide was upregulated in the rat brain after administration of cocaine and amphetamine². CART peptides are suggested to be neuropeptides since they are expressed exclusively in neurons in the brain³. There is much evidence which suggests that CART neuropeptides act through G

protein-coupled receptors, particularly through the Gi/o pathway⁴. The interaction between CART and its receptor(s) leads to a variety of functions. Among the best-known functions modulation of mesolimbic dopamine related reward and reinforcement^{5, 6}, maintenance of energy homeostasis⁶, regulation of the hunger-satiety cycle⁷, modulation of the HPA axis⁸, induction of arousal and alertness⁹, and finally involvement in the cycle of sleep-wake regulation^{7, 9} could be mentioned. Both CART peptides and its mRNA are broadly expressed in the central nervous system¹⁰, while CART mRNA is one of the most plentiful mRNAs in the rat NAc¹¹. Circadian rhythms are internal timekeeping systems, synchronized by environmental cues like light, feeding, and activity, to regulate behavioral and physiological functions. In mammals, the central clock in the suprachiasmatic nucleus (SCN) coordinates peripheral clocks across the body to maintain metabolic and temporal balance¹². Although the SCN was traditionally considered the sole central pacemaker in the mammalian brain, emerging evidence indicates the presence of multiple extra-SCN circadian oscillators in the brain, including the NAc, which drive rhythms in physiology and behavior^{13, 14}. NAc acts as a circadian oscillator, integrating sensory and circadian information to regulate motivated behaviors, with diurnal variations in neuronal activity, dopamine signaling, and molecular clock functions^{15, 16}. Research conducted on rodent models and human post-mortem samples demonstrates substantial circadian rhythmicity within the NAc. Using the PER2:LUC mouse model, ex vivo bioluminescent rhythms were detected in the NAc, supporting findings of diurnal variations in molecular clock gene mRNA and protein expression in the striatum^{17, 18}. RNA-sequencing analyses of human post-mortem NAc tissue reveal robust rhythms in canonical clock genes (e.g., Bmal1, Npas2, Period, Cryptochrome, and Rev-erb α) and enrichment of Circadian Rhythm Signaling pathways among the top rhythmic genes. Functional studies primarily focus on medium spiny neurons (MSNs) and their subtypes, but recent findings also highlight robust circadian rhythms in NAc astrocytes¹⁹.

Having established that the NAc is a site enriched in CART, many studies have been conducted to understand the origin, function, biological properties, and signaling of the intra-NAc of CART peptide. In general, these studies fall into



three categories. A number of studies aim to identify the projection of CART -containing neurons in the NAc. For example, Hubert et al. (2010) demonstrated that the substantia nigra pars reticulata and ventral pallidum are nuclei that receive CART-containing accumbens efferent²⁰. Another part of the studies investigates the changes in the levels of CART and its mRNA in the NAc after treatments/interventions and it also examines the possible mechanisms involved²¹⁻²³. Another group of studies evaluates the effects of intra-NAc administration of the peptide CART and/or its antibody following treatments/interventions²⁴⁻²⁷. Despite the large amount of research being conducted for better understanding of the function of CART in the NAc and other tissues, our knowledge in this area is still in its infancy. However, in spite of our limited knowledge, it is known that CART plays such an important role in biological processes and homeostasis that the body keeps its quantity stable throughout life. Although the average production of the peptide CART remains stable whole life,²⁸ it has been found that this production follows the diurnal circadian rhythm. Reports by Vicentic et al. (2004) suggest that CART levels in peripheral blood exhibit a diurnal rhythm, without clarification of the sources from which circulating CART are derived²⁹. Studies have shown that not only does the expression of CART follow the circadian rhythm, but it is influenced by changes of it. For instance, as circadian rhythm disruptors, sleep deprivation of 10 days in rats noticeably suppressed levels of mRNA for the CART gene both in the arcuate nucleus as well as dorsal hypothalamus³⁰.

The design of the current study was based on the findings of previous studies that showed the property of rhythmicity,²⁹ the effect and also the influenceability of the peptide CART on the circadian rhythm³⁰. The aim of the present study was to determine whether the peptide CART follows a diurnal pattern, specifically in the NAc as CART enriched site, involved in the coordination of the circadian rhythm. Additionally, the study aimed to evaluate how circadian rhythm disruption influences CART mRNA expression in the NAc, providing insights into potential implications for circadian-related disorders and their clinical management.

Materials and Methods

To carry out this experiment, 28 adult male Wistar rats, from 250 to 300 grams, were provided by Animal Centre of Faculty of Medicine in Shahrood University of Medical Sciences. After being transferred to the laboratory, all rats were left undisturbed for one week to acclimate to laboratory conditions. The animals were placed under standard laboratory conditions with room temperature of 21°C and humidity of 50-

45% and were provided with desirable amount of food and water. There was also a 12-hour light/dark cycle for the normal light/dark group (LD) (lights were turned on at 7:00 am and turned off at 7:00 pm) and 24-hour continuous light for the disturbed circadian rhythm group (LL). Rats were maintained according to the guidelines of the National Institutes of Health in the United States. Ethics code is IR.SHMU.REC.1398.058.

To determine the activity/rest pattern, animals were housed in an individual running wheel cage³¹. The baseline activity/rest of the animals was monitored continuously for 30 days on a 12:12 light/dark cycle. Data was collected by a home-made digitized system then visualized and plotted using ImageJ plug-in "ActogramJ". Results revealed that none of the rats needed to be excluded from the study since no one showed abnormal pattern of activity/rest. Therefore, all subjects (n=28) were included in study. Then, half of the total included rats (n=14) were decapitated between Zeitgeber time (ZT) 0–2 (where ZT 0=lights-on and ZT 12=lights-off) for morning groups and ZT 12-14 for evening groups. Animals decapitated³² in the morning were grouped as (LD, morning) and others decapitated in the evening as (LD, evening). Afterward, to model circadian rhythm disruption, the remaining animals (n=14) were exposed to constant light (LL) for 30 consecutive days/nights³³ (from day 31 to 60). On 60th experimental day, half of LL animals (n=7) were decapitated in the morning (LL, Morning) and the other half (n=7) in the evening (LL, Evening). In fact, half of the animals in the LL and LD groups were decapitated in the morning and the other half in the evening to determine the mRNA expression of CART at two time points during the day. Thus, each group (LL and LD) was divided into two groups (morning and evening) based on the time of brain sampling. Four groups (n=7 per group) as follows:

1. (LD, Morning): Rats were left undisturbed for 30 days and remained on a 12/12-h LD cycle. On the 30th day, the animals were decapitated at 7 a.m.
2. (LD, Evening): Rats were left undisturbed for 30 days and remained on a 12/12-h LD cycle. On the 30th day, the animals were decapitated at 7 p.m.
3. (LL, Morning): After 30 days on a 12/12-h LD cycle, the rats were kept in constant light (LL) for 30 days. On day 60, the animals were decapitated at 7 a.m.
4. (LL, Evening): After 30 days of 12/12-h LD cycle, rats were kept in constant light (LL) for 30 days. On the 60th day, the subjects were decapitated at 7 p.m.

The setup of the experiment is shown in Figure 1.



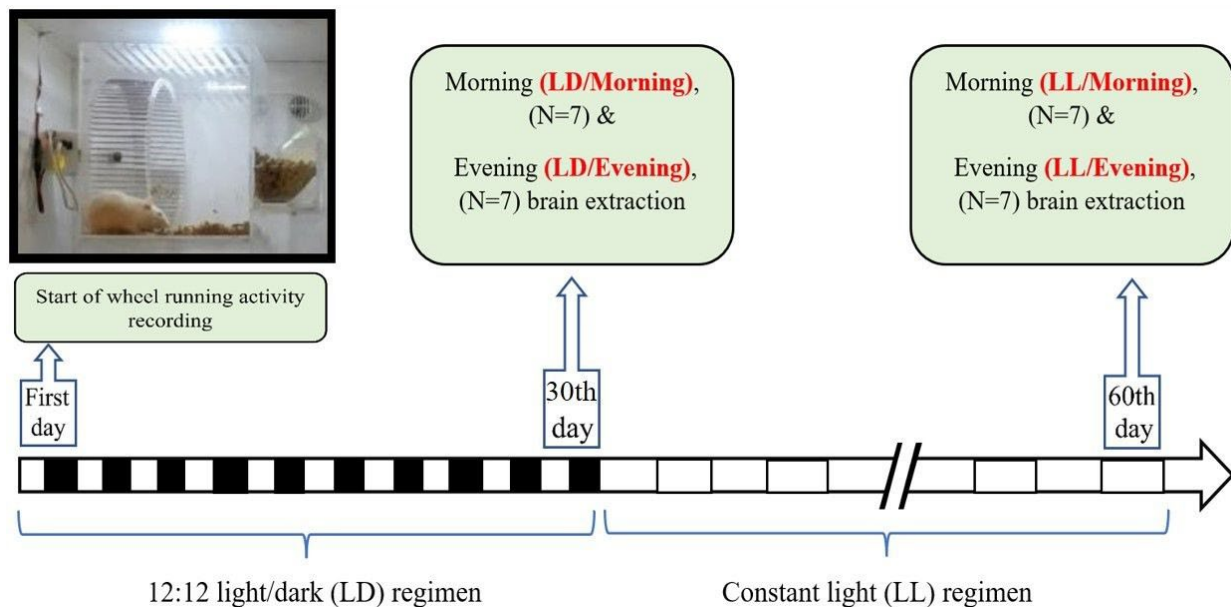


Figure 1. The setup of the experiment (created with biorender.com). Animals were housed individually with voluntary wheel-running devices to monitor baseline activity/rest patterns for 30 days under a 12:12 light/dark (LD) cycle. Data collection and visualization were performed using a digitized system. On the 30th day, 14 rats were randomly assigned to two groups based on decapitation timing: LD Morning (ZT 0–2): Animals were decapitated between 7:00–9:00 a.m. LD Evening (ZT 12–14): Animals were decapitated between 7:00–9:00 p.m.

The remaining 14 rats were exposed to constant light (LL) conditions for 30 consecutive days (Day 31–60) to induce circadian rhythm disruption. On the 60th day the LL animals were divided into two groups based on decapitation timing: LL Morning (ZT 0–2): Decapitated between 7:00–9:00 a.m. LL Evening (ZT 12–14): Decapitated between 7:00–9:00 p.m.

In order to anesthetize animals, Sodium-pentobarbital, 3.5%, 3 ml/kg, was injected intraperitoneally. Using a standard small guillotine, five minutes right after the injection, subject rats were decapitated. Following that, the rat total brain tissue was first extracted and then immersed in extremely cold PBS (phosphate-buffered-saline). Afterward, the tissue was put on a sterile dish that was placed on ice. In order to separate hemispheres from each other, a sagittal cut was first performed and after that NAc were extracted from them. Shortly, a coronal cut of 1 mm was performed in front of the forceps minor of the corpus callosum so as to isolate PFC (cut 1) in accordance with Paxinos and Watson rat brain atlas (2007). Another cut (cut 2) was also done just in front of column of fornix. The area which is between cut 1 and 2 contains NAc. By using a proper device, the NAc was first punched and then isolated. This isolated tissue was placed immediately in individual micro-tubes and immersed quickly in some liquid nitrogen. In final step, after some time, microtubes were transferred to a freezer with -80°C ³⁴.

RNeasy Lipid Tissue Mini Kit (Qiagen) carried out total RNA from samples extraction in accordance with manufacturer's protocol. By a Pico drop Microliter Spectrometer the quantification of extracted RNA was performed and its integrity was also confirmed using gel electrophoresis (1.2% agarose; Gibco/ BRL). 1 μg of extracted RNA was actually reverse-transcribed into first-strand cDNA so as to synthesize complementary DNA (cDNA) by using Qiagen (QuantiTect Reverse Transcription Kit) in a final

volume (20 μl) and then was kept at -20 for further processes ³⁵.

Target gene expression was normalized by beta-actin which acted as the internal standard gene. Both Oligonucleotide primers of real-time PCR amplification of CART as well as beta-actin genes were provided from Qiagen company primer bank.

In all real-time PCR reactions 2 μl cDNA was utilized in a final volume of 25 μl . Power SYBR-Green PCR Master Mix (Life Technologies) was used to complete reactions on StepOnePlus Real-Time PCR System (Applied Biosystems). CART primer pairs' optimized annealing temperature was 61.5. As it was reported in previous sensitization studies, by using beta-actin acting as the reference gene founded data was normalized. The samples were then examined in duplicate and the mean data were also calculated for the following steps. Samples' target gene expression was quantified using standard curve methodology. CART gene's quantity measurement in each sample was first done by defining the cycle where the sample fluorescence met a predetermined threshold significantly above the background. Following that the cycle number was referred to a standard curve which existed in every run of the reaction. The collected data were normalized against beta-actin (the housekeeping gene) for all of observed groups. PCR products specificity was verified by reaching a single peak in melt curve. In order to check the length validation, PCR products were also visualized on 2.5 per cent agarose gel ³⁶.

Statistical analysis data were analyzed using statistic software (Graph Pad version 10). The mean values were calculated in each experimental group. The data were also expressed as: SEM±means (standard error of the mean). A one-way analysis of variance (ANOVA) was used to compare the differences among groups, which was then followed by a post hoc test of Tukey. A value with P-value<0.05 was taken as statistically significant. In order to evaluate noticeable distinctions in relative expression levels between LD and LL group's gene expression experiments data were analyzed using REST (Relative Expression Software Tool- XL version 2). Pair-wise fixed reallocation randomization test is used by the software to determine the importance of results. Gene normalization and quantification alike are also performed at the same time using REST software.

Results

Rest-activity pattern was recorded for 60 consecutive days using wheel running placed on each animal cage. Data was sampled with 3 minutes' intervals. A double plot actogram was then plotted using ImageJ plug-in "ActogramJ" program. The rats whose running-wheel activity rhythms are shown in Figure 2 were exposed to a light-dark cycle for 30 days (LD) and to constant light (250 lux) for 30 days (LL). In the first 30 days' animals exhibited synchronized rest-activity rhythms with light-dark cycle and, in the next 30 days under constant light, free-running rhythms were first shown and then the rhythms deteriorated.

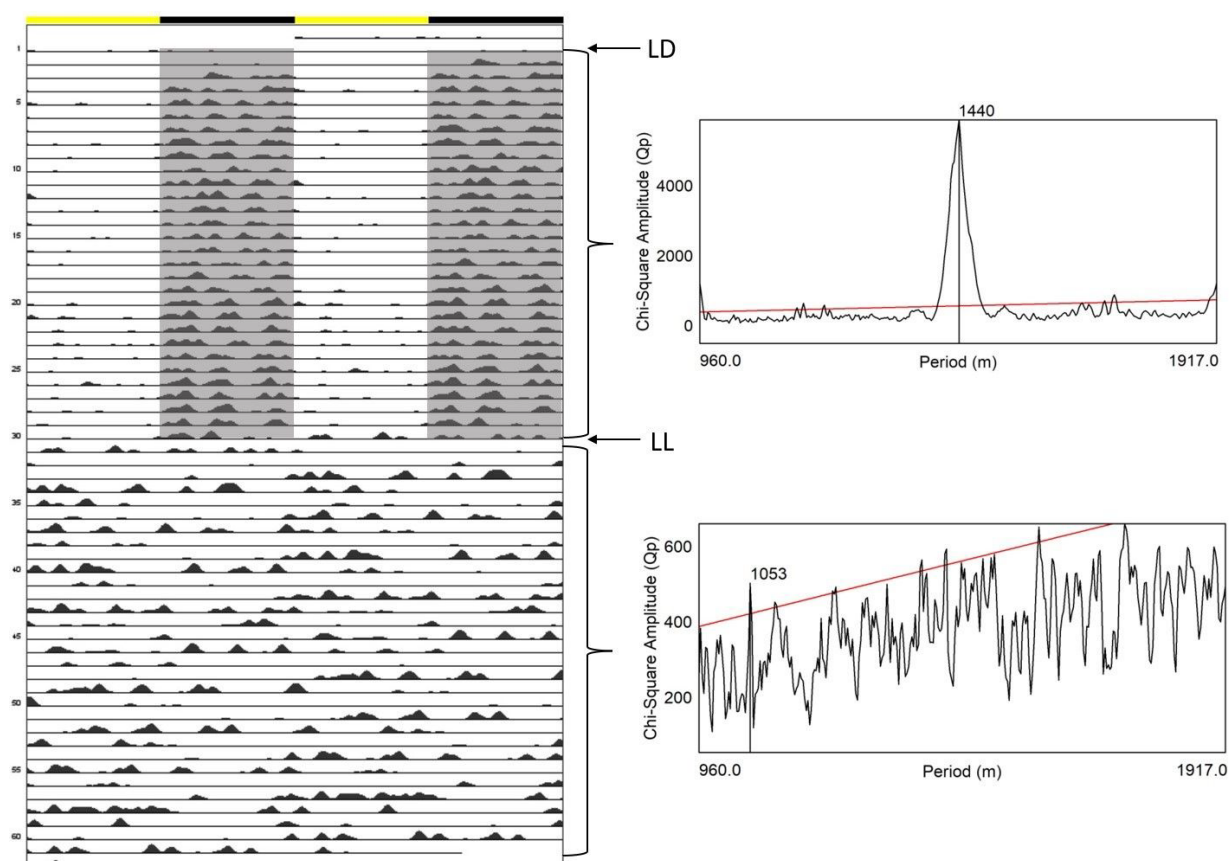


Figure 2. Representative double-plotted actograms of circadian rhythms of wheel running in rat maintained under LD (30 days) and LL (30 days) and corresponding chi square periodogram analyses. Each line of the double-plotted actograms represents 48 h. Dark bars indicate the dark hours and yellow bars indicate the light hours of the light/dark cycle. Dark shadow rectangle indicates aligned phase. chi square periodogram analysis of the LL animals confirmed the absence of significant rhythmic components with a circadian period using a 95% confidence threshold. The circadian rhythm LD rat has a prominent rhythm with a period of 1440 minutes (24 hour).

Statistical analysis of the mRNA level of the gene CART in the NAc region of the brain showed that the level in the LD group was significantly higher in the evening than in the morning (P-value>0.001) (Figure 3. A), similarly, mRNA level of the gene CART was dramatically higher in the animals of the LL group in the evening than in the morning (P-

value>0.01) (Figure 3. B). Further comparisons were made between the animals in the LD group and LL group in the morning and evening. Statistical analysis showed that mRNA level of the CART gene measured in the morning in the LL group was significantly lower than in LD group (P-value>0.05) (Figure 3. C). In addition, the result of the evening examination



of the mRNA level of the CART gene was significantly lower in the NAc of the LL animals than in the LD group (P -

value>0.01) (Figure 3. D).

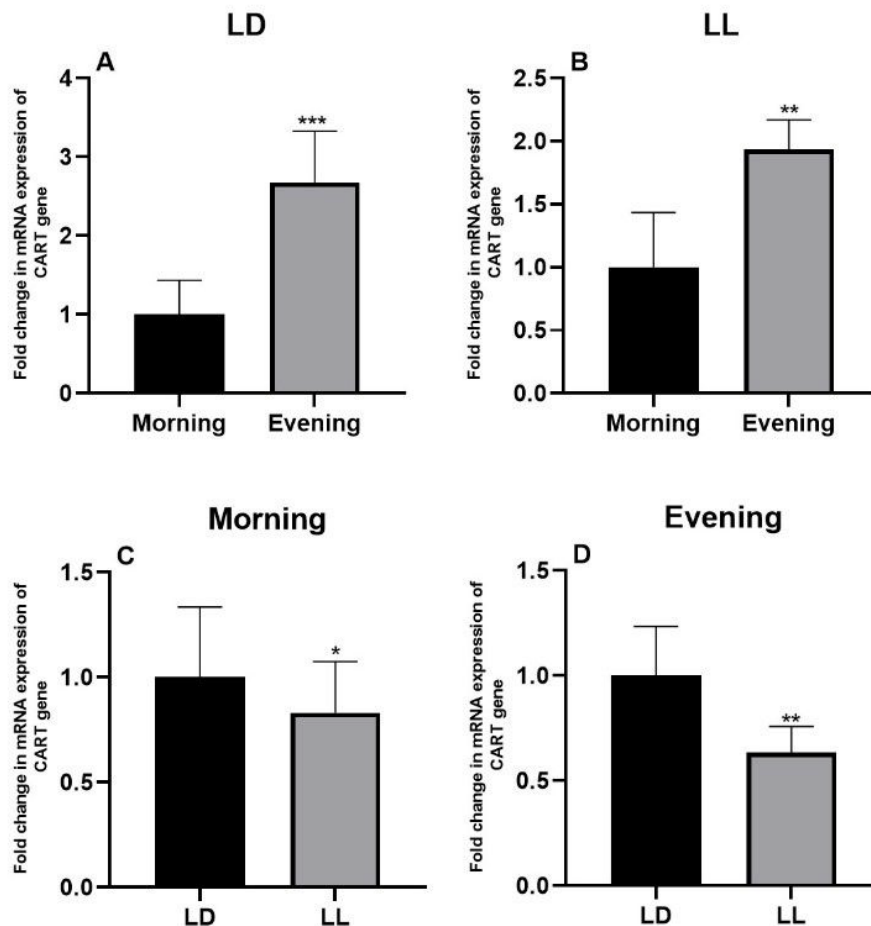


Figure 3. Morning and evening mRNA expression of CART gene (A) in the NAc of LD groups after 10 days' exposure to normal 12-hour light/dark cycle. morning and evening mRNA expression of CART gene (B) in the NAc of LL groups after 30 days' exposure to normal 12-hour light/dark cycle following by 50 days' exposure to constant lighting protocol. Comparison morning mRNA expression of CART gene in the NAc of LD groups and LL animals (C). Comparison evening mRNA expression of CART gene in the NAc of LD groups and LL animals (D). Bars represent fold differences of mean normalized expression values \pm SEM (n=4). ***P-value<0.001, **P-value<0.01, *P-value<0.05

Discussion

The results demonstrated that from day 30 onwards, animals subjected to constant light conditions exhibited disrupted locomotor activity patterns, as observed in double-plotted actograms. These findings highlight the significant role of physical activity in regulating the "circadian syndrome" and disorders associated with circadian rhythms.

In 2019, Pathak et al. demonstrated that mutations in the GCN2 gene in eukaryotes result in alterations in the light/dark cycle, impair nocturnal locomotor behavior, and reduce the rhythmic expression of *per1* and *per2* genes in the SCN³⁷. On the other hand, the disruption in the actogram double-plotted cycle for wheel running in rats with circadian rhythm disorder

can be attributed to the effect of wheel-running exercise on the body clock. This exercise shortens the free-running period, likely due to increased locomotor activity. The mechanism may involve serotonin's role in modulating circadian cycles^{38, 39}. Consistent with previous research, our findings demonstrate that prolonged wheel-running exercise leads to a shortening of the free-running period, particularly under conditions of circadian rhythm disruption. This effect, likely mediated by increased locomotor activity and potentially influenced by serotonin signaling, underscores the complex interplay between physical activity and the regulation of the circadian system. These results suggest that wheel running not only serves as a behavioral adaptation to environmental shifts but also acts as a modulator of intrinsic circadian

mechanisms, with strain-specific and temporal factors contributing to the observed outcomes.

CART expression is known to be modulated by chronic stress and antidepressant interventions, as demonstrated by its downregulation following chronic mild stress⁴⁰ and upregulation after electroconvulsive stimulation in the nucleus accumbens⁴¹. Moreover, administration of exogenous CART peptides has been shown to reduce immobility time in the forced swim test, supporting its antidepressant-like effects⁴². Recent evidence also suggests that *CART* gene may serve as a molecular marker for antidepressant response, particularly in ketamine-treated mice, where increased CART expression in the anterior cingulate cortex correlates with behavioral improvement⁴³. These findings underscore the potential involvement of CART in mood regulation.

The current study has revealed that mRNA expression of CART peptides in NAc follows circadian rhythms. In addition, our measurement of CART peptide mRNA levels shows that its levels are about 2 times higher in the evening than in the morning. It is important to point out that mice and rats are nocturnal rodents, i.e., they are active during the dark period and rest during the day⁴⁴. Therefore, the higher level of CART peptide mRNA in the evening reflects the stronger gene expression during the active/dark phase of the rat compared with the inactive/light phase. The main finding of the present study is that the disruption of circadian rhythms by continuous light leads to a down-regulation of CART both in the morning and in the evening. Interestingly, our study has shown that CART is still subject to circadian control despite the reduced expression under continuous light. This diurnal rhythm, which was observed under constant light conditions, suggests the endogenous nature of this modulation even in the absence of the light/dark cycle as an external cue. However, it is not clear whether continued disruption of the circadian rhythm can disrupt the resilience of endogenous regulation in the long term. Further research is needed to clarify this question. Significant rhythmicizing of various gene expressions and/or protein productions in NAc neurons has been addressed several times in the literature⁴⁵. Studies in rodents have shown that concentrations of dopamine and its metabolites in the NAc exhibit robust diurnal rhythms⁴⁶⁻⁴⁹. In addition, diurnal variations in Cryptochrome protein levels in the NAc have been reported⁵⁰. Diurnal variation in the NAc is also shown in core clock genes which include *Npas2*, *Clock* as well as *Period*⁵¹. In addition to *Npas2* and *Bmal1*, *Rev-erb α* and *ROR α* as their transcriptional regulators indicate remarkable diurnal variation in the NAc, too. This circadian regulation reaches the regulation of *Sirt1*, *Nampt*, and also, the level of the mitochondrial coenzyme NAD⁺, all of which show diurnal variations in NAc⁵². Surprisingly, this is also evident in gene expression analysis at the time of death in human postmortem tissue, where all canonical clock genes including *Bmal1*, *Npas2*, *Period*, *Cryptochrome*, and *Rev-erb α* are strongly rhythmic in NAc from healthy donors^{53,54}. A study which has been published recently has found that robust rhythms are also shown in NAc astrocytes in particular⁵⁵. After using next generation total RNA sequencing in NAc astrocytes across diurnal time, it was found that not only do NAc astrocytes express robust rhythms in canonical clock genes unanimously, but also have significant diurnal rhythms in approximately 43% of the total NAc astrocyte transcriptome⁵⁵. Together with

previous findings of neuronal rhythmicity, this lately characterized astrocyte-specific rhythmicity underscores the complicated nature of circadian regulation in NAc cells. Focus of previous studies mentioned above has been on the rhythmicity of clock genes in NAc, and a limited number of studies address the rhythmicity of expression of other molecules. However, with increasing evidence for the existence of rhythmicity in expression of genes, there is still the possibility of discovering other circadian oscillators in the NAc as extra-SCN circadian oscillators region. We found that expression of the *CART* gene (as a non-clock gene) in rat NAc is subject to diurnal variation. Our results support the further studies which have shown that CART follows a circadian pattern in blood and brain of rats alike^{29,56}. It should be acknowledged that *CART* gene promoter contains an E-box⁵⁷ being implicated in circadian rhythmicity;⁵⁸ hence, the regulation of expression, based on a daily cycle, is not entirely surprising. However, the pattern of CART expression rhythmicity in cells of various brain regions may be different. Therefore, the determination of these different rhythms may be valuable for the discovery of the biological and pharmacological functional roles in which the peptide CART is involved.

The circadian regulation of CART expression in the nucleus accumbens appears to parallel the rhythmic profile of CART peptide levels and daily corticosterone secretion^{59,60}. Evidence suggests that CART expression may be modulated by core clock genes such as *Per1*, which influences time-dependent behavioral responses to psychostimulants⁶¹. Notably, disruptions in *Per1* expression have been linked to altered reward sensitivity, implying a potential regulatory link between circadian genes and CART-mediated processes. Moreover, interactions between *Per1* and NMDA receptor signaling pathways may further contribute to light-induced disturbances in CART rhythmicity⁶². These findings suggest that constant light exposure may interfere with CART expression through dysregulation of clock gene networks and associated neurotransmitter systems.

Previous studies have shown that the diurnal rhythm of CART expression is sensitive to metabolic status, feeding cues, and circadian regulation. In regular-fed animals, CART levels in the nucleus accumbens (NAc) display a robust diurnal rhythm that is abolished under fasting conditions⁵⁹. This disruption may be mediated in part by leptin signaling, as fasting reduces circulating leptin levels and the CART promoter contains a STAT-binding site responsive to leptin^{63,64}. Leptin crosses the blood-brain barrier⁶⁵ and regulates anorexigenic peptides, including CART, in hypothalamic circuits involved in energy balance⁶⁶. CART's physiological role in appetite regulation has been demonstrated by multiple studies: immunoneutralization of CART increases food intake^{67,68}, and global CART knockout leads to hyperphagia and weight gain^{69,70}. Moreover, CART neurons in the hindbrain suppress feeding behavior through projections to the hypothalamus⁷¹, and CART signaling in the nucleus tractus solitarius (NTS) is critical for satiety⁷². Disrupted circadian rhythms have been linked to overeating, weight gain, and obesity⁷³⁻⁷⁵, and reduced CART expression in SCN has been observed in clock gene mutant rodents with associated metabolic dysregulation⁷⁶. In our study, constant light exposure disrupted CART rhythmicity, particularly in the NAc



brain region implicated in both circadian and feeding circuits. Collectively, these findings suggest that reduced CART expression following circadian disruption may promote hyperphagia and contribute to metabolic disturbances. Future investigations should assess the causal role of CART in circadian rhythm-induced obesity, particularly in mesolimbic reward pathways.

Ethical Considerations

This article was approved by the ethical committee of Shahrood University of Medical Sciences, Iran (Ethical Code: IR.SHMU.REC.1398.058).

Acknowledgment

The authors are grateful to the research assistant of Shahrood university of medical sciences and the respected participants who collaborated in this research.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Funding

The authors themselves covered all costs associated with this study. No external funding or financial assistance was received.

References

- Spieß J, Villarreal J, Vale W. Isolation and sequence analysis of a somatostatin-like polypeptide from ovine hypothalamus. *Biochemistry*. 1981;20(7):1982-1988. doi: 10.1021/bi00510a038
- Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *Journal of Neuroscience*. 1995;15(3):2471-2481. doi: 10.1523/JNEUROSCI.15-03-02471.1995
- Kuhar MJ, Vechia SED. CART peptides: novel addiction-and feeding-related neuropeptides. *Trends in neurosciences*. 1999;22(7):316-320. doi: 10.1016/S0166-2236(98)01377-0
- Vicentic A, Lakatos A, Jones D. The CART receptors: background and recent advances. *Peptides*. 2006;27(8):1934-1937. doi: 10.1016/j.peptides.2006.03.031
- Bakhtazad A, Vouseoghi N, Garmabi B, Zarrindast MR. Evaluation of CART peptide level in rat plasma and CSF: Possible role as a biomarker in opioid addiction. *Peptides*. 2016;84:1-6. doi: 10.1016/j.peptides.2016.06.010
- Philpot K, Smith Y. CART peptide and the mesolimbic dopamine system. *Peptides*. 2006;27(8):1987-1992. doi: 10.1016/j.peptides.2005.11.028
- Méndez-Díaz M, Martín ED, Morales MP, Ruiz-Contreras A, Navarro L, Prospéro-García O. The anorexigenic peptide cocaine-and-amphetamine-regulated transcript modulates rem-sleep in rats. *Neuropeptides*. 2009;43(6):499-505. doi: 10.1016/j.npep.2009.08.004
- Stanek LM. Cocaine-and amphetamine related transcript (CART) and anxiety. *Peptides*. 2006;27(8):2005-2011. doi: 10.1016/j.peptides.2006.01.027
- Keating GL, Kuhar MJ, Bliwise DL, Rye DB. Wake promoting effects of cocaine and amphetamine-regulated transcript (CART). *Neuropeptides*. 2010;44(3):241-246. doi: 10.1016/j.npep.2009.12.013
- Ahmadian-Moghadam H, Sadat-Shirazi M-S, Zarrindast M-R. Cocaine-and amphetamine-regulated transcript (CART): a multifaceted neuropeptide. *Peptides*. 2018;110:56-77. doi: 10.1016/j.peptides.2018.10.008
- Hurd YL, Fagergren P. Human cocaine-and amphetamine-regulated transcript (CART) mRNA is highly expressed in limbic-and sensory-related brain regions. *Journal of Comparative Neurology*. 2000;425(4):583-598. doi: 10.1002/1096-9861(20001002)425:4<583::AID-CNE8>3.0.CO;2-#
- Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. 2010;72(1):517-549. doi: 10.1146/annurev-physiol-021909-135821
- Begemann K, Neumann AM, Oster H. Regulation and function of extra-SCN circadian oscillators in the brain. 2020;229(1):e13446. doi: 10.1111/apha.13446
- Becker-Krail DD, Walker WH, Nelson RJ. The ventral tegmental area and nucleus accumbens as circadian oscillators: implications for drug abuse and substance use disorders. 2022;13:886704. doi: 10.3389/fphys.2022.886704
- Parekh PK, Becker-Krail D, Sundaravelu P, et al. Altered GluA1 (Gria1) Function and Accumbal Synaptic Plasticity in the ClockΔ19 Model of Bipolar Mania. *Biological psychiatry*. Dec 1 2018;84(11):817-826. doi: 10.1016/j.biopsych.2017.06.022
- Alonso IP, Pino JA, Kortagere S, Torres GE, España RA. Dopamine transporter function fluctuates across sleep/wake state: potential impact for addiction. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Mar 2021;46(4):699-708. doi: 10.1038/s41386-020-00879-2
- Landgraf D, Long JE, Welsh DK. Depression-like behaviour in mice is associated with disrupted circadian rhythms in nucleus accumbens and periaqueductal grey. *The European journal of neuroscience*. May 2016;43(10):1309-20. doi: 10.1111/ejn.13085
- Logan RW, Edgar N, Gillman AG, Hoffman D, Zhu X, McClung CA. Chronic Stress Induces Brain Region-Specific Alterations of Molecular Rhythms that Correlate with Depression-like Behavior in Mice. *Biological psychiatry*. Aug 15 2015;78(4):249-58. doi: 10.1016/j.biopsych.2015.01.011
- Becker-Krail DD, Ketchesin KD, Burns JN, et al. Astrocyte molecular clock function in the nucleus accumbens is important for reward-related behavior. 2022;92(1):68-80. doi: 10.1016/j.biopsych.2022.02.007
- Hubert G, Manvich D, Kuhar M. Cocaine and amphetamine-regulated transcript-containing neurons in the nucleus accumbens project to the ventral pallidum in the rat and may inhibit cocaine-induced locomotion. *Neuroscience*. 2010;165(1):179-187. doi: 10.1016/j.neuroscience.2009.10.013
- Jean A, Conduetier G, Manrique C, et al. Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. *Proceedings of the National Academy of Sciences*. 2007;104(41):16335-16340. doi: 10.1073/pnas.0701471104
- Salinas A, Wilde JD, Maldve RE. Ethanol enhancement of cocaine-and amphetamine-regulated transcript mRNA and peptide expression in the nucleus accumbens. *Journal of neurochemistry*. 2006;97(2):408-415. doi: 10.1111/j.1471-4159.2006.03745.x
- Hubert G, Kuhar M. Cocaine administration increases the fraction of CART cells in the rat nucleus accumbens that co-immunostain for c-Fos. *Neuropeptides*. 2008;42(3):339-343. doi: 10.1016/j.npep.2008.01.001
- Bakhtazad A, Vouseoghi N, Nasehi M, Sanadgol N, Garmabi B, Zarrindast MR. The effect of microinjection of CART 55-102 into the nucleus accumbens shell on morphine-induced conditioned place preference in rats: Involvement of the NMDA receptor. *Peptides*. 2020;129:170319. doi: 10.1016/j.peptides.2020.170319
- Awathale SN, Choudhary AG, Subhedar NK, Kokare DM. Neuropeptide CART modulates dopamine turnover in the nucleus accumbens: Insights into the anatomy of rewarding circuits. *Journal of Neurochemistry*. 2021;158(5):1172-1185. doi: 10.1111/jnc.15479
- Fu Q, Zhou X, Dong Y, et al. Decreased caffeine-induced locomotor activity via microinjection of CART peptide into the nucleus accumbens is linked to inhibition of the pCaMKIIα-D3R interaction. *Plos one*. 2016;11(7):e0159104. doi: 10.1371/journal.pone.0159104
- Jaworski JN, Hansen ST, Kuhar MJ, Mark GP. Injection of CART (cocaine-and amphetamine-regulated transcript) peptide into the nucleus accumbens reduces cocaine self-administration in rats. *Behavioural brain research*. 2008;191(2):266-271. doi: 10.1016/j.bbr.2008.03.039
- Armbruszt S, Figler M, Ábrahám H. Stability of CART peptide expression in the nucleus accumbens in aging. *Acta Biologica Hungarica*. 2015;66(1):1-13. doi: 10.1556/ABiol.66.2015.1.1
- Vicentic A, Dominguez G, Hunter R, Philpot K, Wilson M, Kuhar M. Cocaine-and amphetamine-regulated transcript peptide levels in blood exhibit a diurnal rhythm: regulation by glucocorticoids. *Endocrinology*. 2004;145(9):4119-4124. doi: 10.1210/en.2003-1648
- Baldo BA, Hanlon EC, Obermeyer W, Bremer Q, Paletz E, Benca RM. Upregulation of gene expression in reward-modulatory striatal opioid systems



- by sleep loss. *Neuropsychopharmacology*. 2013;38(13):2578-2587. doi: 10.1038/npp.2013.174
31. Rea MS, Figueiro MG. Quantifying light-dependent circadian disruption in humans and animal models. *Chronobiology International*. 2014/12/01 2014;31(10):1239-1246. doi: 10.3109/07420528.2014.957302
32. Rafaiee R, Mohseni FJJoME, Medicine Ho. Ethical considerations and challenges associated with euthanasia in laboratory animal research. 2024;17. doi: 10.18502/jmehm.v17i11.18663
33. Morimoto Y, Oishi T, Arisue K, Yamamura Y. Effect of food restriction and its withdrawal on the circadian adrenocortical rhythm in rats under constant dark or constant lighting condition. *Neuroendocrinology*. 1979;29(2):77-83. doi: 10.1159/000122908
34. Bakhtazad A, Voussooghi N, Garmabi B, Zarrindast M. CART peptide and opioid addiction: expression changes in male rat brain. *Neuroscience*. 2016;325:63-73. doi: 10.1016/j.neuroscience.2016.02.071
35. Garmabi B, Voussooghi N, Vosough M, Yoonessi A, Bakhtazad A, Zarrindast M. Effect of circadian rhythm disturbance on morphine preference and addiction in male rats: involvement of period genes and dopamine D1 receptor. *Neuroscience*. 2016;322:104-114. doi: 10.1016/j.neuroscience.2016.02.019
36. Bakhtazad A, Voussooghi N, Garmabi B, Zarrindast MR. Evaluation of the CART peptide expression in morphine sensitization in male rats. *European journal of pharmacology*. 2017;802:52-59. doi: 10.1016/j.ejphar.2017.02.040
37. Pathak SS, Liu D, Li T, et al. The eIF2 α kinase GCN2 modulates period and rhythmicity of the circadian clock by translational control of Atf4. 2019;104(4):724-735. e6. doi: 10.1016/j.neuron.2019.08.007
38. Oneda S, Cao S, Haraguchi A, Sasaki H, Shibata SJFiP. Wheel-running facilitates phase advances in locomotor and peripheral circadian rhythm in social jet lag model mice. 2022;13:821199. doi: 10.3389/fphys.2022.821199
39. Mohammadi S, Zahmatkesh M, Asgari Y, Aminyavari S, Hassanzadeh G. Evaluation of hippocampal arylalkylamine N-acetyltransferase activity in amyloid- β neurotoxicity. *Journal of molecular endocrinology*. Aug 1 2023;71(2). doi: 10.1530/JME-22-0161
40. Orsetti M, Di Brisco F, Canonico PL, Genazzani AA, Ghi P. Gene regulation in the frontal cortex of rats exposed to the chronic mild stress paradigm, an animal model of human depression. *The European journal of neuroscience*. Apr 2008;27(8):2156-64. doi: 10.1111/j.1460-9568.2008.06155.x
41. Roh MS, Cui FJ, Ahn YM, Kang UG. Up-regulation of cocaine- and amphetamine-regulated transcript (CART) in the rat nucleus accumbens after repeated electroconvulsive shock. *Neuroscience research*. Oct 2009;65(2):210-3. doi: 10.1016/j.neures.2009.06.013
42. Dandekar MP, Singru PS, Kokare DM, Subhedar NK. Cocaine- and amphetamine-regulated transcript peptide plays a role in the manifestation of depression: social isolation and olfactory bulbectomy models reveal unifying principles. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Apr 2009;34(5):1288-300. doi: 10.1038/npp.2008.201
43. Funayama Y, Li H, Ishimori E, et al. Antidepressant Response and Stress Resilience Are Promoted by CART Peptides in GABAergic Neurons of the Anterior Cingulate Cortex. *Biological psychiatry global open science*. Jan 2023;3(1):87-98. doi: 10.1016/j.bpsgos.2021.12.009
44. Hart J. *Rodents. Mammals*. Elsevier; 1971:1-149. doi: 10.1016/B978-0-12-747602-5.50007-1
45. Becker-Krail DD, Walker WH, Nelson RJ. The Ventral Tegmental Area and Nucleus Accumbens as Circadian Oscillators: Implications for Drug Abuse and Substance Use Disorders. *Frontiers in Physiology*. 2022;762. doi: 10.3389/fphys.2022.886704
46. Alonso I, Pino J, Kortagere S, Torres G, España R. Dopamine transporter function fluctuates across sleep/wake state: potential impact for addiction. *Neuropsychopharmacology*. 2021;46(4):699-708. doi: 10.1038/s41386-020-00879-2
47. Koch C, Begemann K, Kiehn J, et al. Circadian regulation of hedonic appetite in mice by clocks in dopaminergic neurons of the VTA. *Nature communications*. 2020;11(1):1-11. doi: 10.1038/s41467-020-16882-6
48. Ferris MJ, España RA, Locke JL, et al. Dopamine transporters govern diurnal variation in extracellular dopamine tone. *Proceedings of the National Academy of Sciences*. 2014;111(26):E2751-E2759. doi: 10.1073/pnas.1407935111
49. Castaneda TR, de Prado BM, Prieto D, Mora F. Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. *Journal of pineal research*. 2004;36(3):177-185. doi: 10.1046/j.1600-079X.2003.00114.x
50. Porcu A, Vaughan M, Nilsson A, Arimoto N, Lamia K, Welsh DK. Vulnerability to helpless behavior is regulated by the circadian clock component CRYPTOCHROME in the mouse nucleus accumbens. *Proceedings of the National Academy of Sciences*. 2020;117(24):13771-13782. doi: 10.1073/pnas.2000258117
51. Falcon E, Ozburn A, Mukherjee S, Roybal K, McClung CA. Differential regulation of the period genes in striatal regions following cocaine exposure. *PLoS One*. 2013;8(6):e66438. doi: 10.1371/journal.pone.0066438
52. Becker-Krail DD, Parekh PK, Ketchesin KD, et al. Circadian transcription factor NPAS2 and the NAD $^{+}$ -dependent deacetylase SIRT1 interact in the mouse nucleus accumbens and regulate reward. *European Journal of Neuroscience*. 2022;55(3):675-693. doi: 10.1111/ejn.15596
53. Ketchesin KD, Zong W, Hildebrandt MA, et al. Diurnal rhythms across the human dorsal and ventral striatum. *Proceedings of the National Academy of Sciences*. 2021;118(2):e2016150118. doi: 10.1073/pnas.2016150118
54. Li JZ, Bunney BG, Meng F, et al. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proceedings of the National Academy of Sciences*. 2013;110(24):9950-9955. doi: 10.1073/pnas.1305814110
55. Becker-Krail DD, Ketchesin KD, Burns JN, et al. Astrocyte Molecular Clock Function in the Nucleus Accumbens Is Important for Reward-Related Behavior. *Biological Psychiatry*. 2022. doi: 10.1016/j.biopsych.2022.02.007
56. Vicentic A, Lakatos A, Hunter R, Philpot K, Dominguez G, Kuhar MJ. CART peptide diurnal rhythm in brain and effect of fasting. *Brain research*. 2005;1032(1-2):111-115. doi: 10.1016/j.brainres.2004.10.053
57. Dominguez G, Lakatos A, Kuhar MJ. Characterization of the cocaine- and amphetamine-regulated transcript (CART) peptide gene promoter and its activation by a cyclic AMP-dependent signaling pathway in GH3 cells. *Journal of neurochemistry*. 2002;80(5):885-893. doi: 10.1046/j.0022-3042.2002.00775.x
58. Gekakis N, Staknis D, Nguyen HB, et al. Role of the CLOCK protein in the mammalian circadian mechanism. *Science*. 1998;280(5369):1564-1569. doi: 10.1126/science.280.5369.1564
59. Vicentic A, Lakatos A, Hunter R, Philpot K, Dominguez G, Kuhar MJ. CART peptide diurnal rhythm in brain and effect of fasting. *Brain research*. Jan 25 2005;1032(1-2):111-5. doi: 10.1016/j.brainres.2004.10.053
60. Jaworski JN, Vicentic A, Hunter RG, Kimmel HL, Kuhar MJ. CART peptides are modulators of mesolimbic dopamine and psychostimulants. *Life sciences*. Jun 27 2003;73(6):741-7. doi: 10.1016/S0024-3205(03)00394-1
61. Abarca C, Albrecht U, Spanagel R. Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proceedings of the National Academy of Sciences of the United States of America*. Jun 25 2002;99(13):9026-30. doi: 10.1073/pnas.142039099
62. Nikaido T, Akiyama M, Moriya T, Shibata S. Sensitized increase of period gene expression in the mouse caudate/putamen caused by repeated injection of methamphetamine. *Molecular pharmacology*. Apr 2001;59(4):894-900. doi: 10.1016/S0026-895X(24)09259-9
63. Elimam A, Marcus C. Meal timing, fasting and glucocorticoids interplay in serum leptin concentrations and diurnal profile. *European journal of endocrinology*. Aug 2002;147(2):181-8. doi: 10.1053/eje.0.1470181
64. Dominguez G, Lakatos A, Kuhar MJ. Characterization of the cocaine- and amphetamine-regulated transcript (CART) peptide gene promoter and its activation by a cyclic AMP-dependent signaling pathway in GH3 cells. *Journal of neurochemistry*. Mar 2002;80(5):885-93. doi: 10.1046/j.0022-3042.2002.00775.x
65. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. *Peptides*. 1996;17(2):305-11. doi: 10.1016/0196-9781(96)00025-3
66. Wanlong Z, Di Z, Dongmin H, Guang YJTEZJ. Roles of hypothalamic neuropeptide gene expression in body mass regulation in *Ethionomys miletus* (Mammalia: Rodentia: Cricetidae). 2017;84(1):322-333. doi: 10.1080/24750263.2017.1334840
67. Kristensen P, Judge ME, Thim L, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature*. May 7 1998;393(6680):72-6. doi: 10.1038/29993
68. Lambert PD, Couceyro PR, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ. CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse (New York, NY)*. Aug 1998;29(4):293-8. doi: 10.1002/(SICI)1098-2396(199808)29:4<293::AID-SYN1>3.0.CO;2-0



69. Asnicar MA, Smith DP, Yang DD, et al. Absence of cocaine- and amphetamine-regulated transcript results in obesity in mice fed a high caloric diet. *Endocrinology*. Oct 2001;142(10):4394-400. doi: [10.1210/en.142.10.4394](https://doi.org/10.1210/en.142.10.4394)
70. Wierup N, Richards WG, Bannan AW, Kuhar MJ, Ahrén B, Sundler F. CART knock out mice have impaired insulin secretion and glucose intolerance, altered beta cell morphology and increased body weight. *Regulatory peptides*. Jul 15 2005;129(1-3):203-11. doi: [10.1016/j.regpep.2005.02.016](https://doi.org/10.1016/j.regpep.2005.02.016)
71. Skibicka KP, Alhadeff AL, Grill HJ. Hindbrain cocaine- and amphetamine-regulated transcript induces hypothermia mediated by GLP-1 receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. May 27 2009;29(21):6973-81. doi: [10.1523/JNEUROSCI.6144-08.2009](https://doi.org/10.1523/JNEUROSCI.6144-08.2009)
72. Lee SJ, Krieger JP, Vergara M, et al. Blunted Vagal Cocaine- and Amphetamine-Regulated Transcript Promotes Hyperphagia and Weight Gain. *Cell reports*. Feb 11 2020;30(6):2028-2039.e4. doi: [10.1016/j.celrep.2020.01.045](https://doi.org/10.1016/j.celrep.2020.01.045)
73. Bray MS, Young ME. Circadian rhythms in the development of obesity: potential role for the circadian clock within the adipocyte. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. Mar 2007;8(2):169-81. doi: [10.1111/j.1467-789X.2006.00277.x](https://doi.org/10.1111/j.1467-789X.2006.00277.x)
74. Broussard JL, Van Cauter E. Disturbances of sleep and circadian rhythms: novel risk factors for obesity. *Current opinion in endocrinology, diabetes, and obesity*. Oct 2016;23(5):353-9. doi: [10.1097/MED.0000000000000276](https://doi.org/10.1097/MED.0000000000000276)
75. Rácz B, Dušková M, Stárka L, Hainer V, Kunešová M. Links between the circadian rhythm, obesity and the microbiome. *Physiological research*. Nov 28 2018;67(Suppl 3):S409-s420. doi: [10.33549/physiolres.934020](https://doi.org/10.33549/physiolres.934020)
76. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science (New York, NY)*. May 13 2005;308(5724):1043-5. doi: [10.1126/science.1108750](https://doi.org/10.1126/science.1108750)

