PLOS ONE

RESEARCH ARTICLE

Pharmacological and Genetic Modulation of REV-ERB Activity and Expression Affects Orexigenic Gene Expression

Ariadna Amador[1], Yongjun Wang[2], Subhashis Banerjee[2], Theodore M. Kameneka[1], Laura A. Solt[1], Thomas P. Burris[2]*

1 Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, Florida, United States of America, **2** Department of Pharmacology and Physiology, Saint Louis University, Saint Louis, Missouri, United States of America

* burristp@slu.edu

Citation: Amador A, Wang Y, Banerjee S, Kameneka TM, Solt LA, Burris TP (2016) Pharmacological and Genetic Modulation of REV-ERB Activity and Expression Affects Orexigenic Gene Expression. PLoS ONE 11(3): e0151014. doi:10.1371/journal.pone.0151014

Editor: Nicolas Cermakian, McGill University, CANADA

Received: November 12, 2015

Accepted: February 23, 2016

Published: March 10, 2016

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by the National Institute of Mental Health (MH09342). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The nuclear receptors REV-ERBα and REV-ERBβ are transcription factors that play pivotal roles in the regulation of the circadian rhythm and various metabolic processes. The circadian rhythm is an endogenous mechanism, which generates entrainable biological changes that follow a 24-hour period. It regulates a number of physiological processes, including sleep/wakeful cycles and feeding behaviors. We recently demonstrated that REV-ERB-specific small molecules affect sleep and anxiety. The orexinergic system also plays a significant role in mammalian physiology and behavior, including the regulation of sleep and food intake. Importantly, orexin genes are expressed in a circadian manner. Given these overlaps in function and circadian expression, we wanted to determine whether the REV-ERBs might regulate orexin. We found that acute *in vivo* modulation of REV-ERB activity, with the REV-ERB-specific synthetic ligand SR9009, affects the circadian expression of orexinergic genes in mice. Long term dosing with SR9009 also suppresses orexinergic gene expression in mice. Finally, REV-ERBβ-deficient mice present with increased orexinergic transcripts. These data suggest that the REV-ERBs may be involved in the repression of orexinergic gene expression.

Introduction

The circadian rhythm is an autonomous, 24-hour, self-sustained oscillation regulating the physiology and behavior of organisms ranging from bacteria to humans [1–7]. The master clock is located in the suprachiasmatic nucleus (SCN) in the hypothalamus and is maintained by a negative feedback loop in which a CLOCK-BMAL1 complex activates E-box containing genes, including *Cryptochrome* (*Cry1* and *Cry2*), and *Period* (*Per1*, *Per2*, and *Per3*). In turn, the CRY-PER complex inhibits the activating action of the CLOCK-BMAL1 heterodimers. The REV-ERBs have been demonstrated to be an essential part of the accessory loop by inhibiting the transcription of the core cock genes *BMAL1*, *NPAS2*, and *CLOCK*. [8–13]. The molecular oscillations that occur in the brain also occur in peripheral organs and are involved in the regulation of metabolic functions [12, 14–16].

Recent data from our laboratory have demonstrated that the REV-ERBs are also involved in the maintenance of sleep architecture and anxiety [17, 18]. Using SR9009, a REV-ERB-specific synthetic agonist, we demonstrated that acute intraperitoneal injections at Zeitgeber time (ZT) 6 induced wakefulness during the subjective night period while chronic agonist administration reduced anxiety-like behaviors in mice. Conversely, mice deficient in REV-ERBβ displayed enhanced anxiety in different behavioral paradigms [19]. In a separate study, Jager *et al* demonstrated that mice lacking *REV-ERBα* displayed a hyperactive phenotype and decreased habituation in novel object paradigms as well as impaired short- and long-term memory. This behavior was determined to be due to direct regulation of tyrosine hydroxylase by REV-ERBα [20].

Extensive work has highlighted the importance of the lateral hypothalamic area (LHA) in wakefulness, feeding behavior, and energy metabolism [21, 22]. Prepro-orexin [PPO, (*Hcrt*)], also known as hypocretin, is a precursor neuropeptide, which generates orexin-A (hypocretin-1) and orexin-B (hypocretin-2). Orexin-A and -B peptides are exclusively produced in the lateral (perifornical region) and dorsal hypothalamus [23–28] and bind the G-protein coupled orexin 1 and 2 receptors, (OX_1R and OX_2R), respectively, also known as Hypocretin-1 Receptor (*HCRTR1*) and Hypocretin-2 Receptor (*HCRTR2*). The orexin peptides have been shown to play key roles in sleep, energy metabolism and feeding [22, 29–32]. In the brain, orexin signaling, particularly via OX_1R, is involved in reward behavior, related to feeding, and drugs of abuse, while orexinergic signaling via OX_2R is classically involved in wake maintenance [23, 33–39]. *Hcrt* has been previously shown to display a circadian pattern of expression during a 24h cycle in the hypothalamus [40]. Hypothalamic orexin neurons receive and extend projections to and receive projections from various regions in the brain, including the SCN [41]. The orexinergic neurons extending from the hypothalamus also innervate wake centers in the brainstem, including the locus coeruleus and the raphe nuclei, centers for sleep/wake regulation [25], and the peripheral autonomic nervous system [27]. Strikingly, mice lacking orexin are narcoleptic and develop late-onset obesity related to decreased energy expenditure [24, 42], supporting a key role for orexin in sleep-wake cycles and control of metabolic activity. Interestingly, circadian disruptions occurring due to mutations in core clock components, including the *Clock* gene, alter day-night expression levels of hypothalamic peptides including orexin [40], suggesting a link between orexin and the master clock. Moreover, orexin expression has been demonstrated to be expressed in a circadian manner [40]. Finally, the orexin pathway is thought to be involved in psychiatric disorders, such as anxiety, panic, depression, and schizophrenia, although very little is still known about its role [43–50].

Given the correlative links between the orexinergic and REV-ERB systems, specifically the regulation of circadian behaviors and metabolism, we investigated whether these two signaling pathways may converge and interact to regulate physiological processes. We demonstrate that REV-ERBs influence the transcription of the orexinergic genes in the hypothalamus and other centers in the brain. Given the intrinsic negative side effects associated with excessive motivation for food and lack of sleep, including anxiety, obesity, diabetes, and low self-esteem, our experiments suggest that the REV-ERB regulation of the orexin pathway may hold utility in ameliorating the detrimental effects of imbalanced circadian behavior via orexinergic pathways.

Materials and Methods

Mice

Male C57BL/6 mice, 8–10 weeks old, were obtained from Jackson laboratories (Bar Harbor, ME). *REV-ERBβ* KO mice were generated by breeding *REV-ERBβ* floxed mice ($Nr1d2^{fl/fl}$) with *EIIa-Cre* mice obtained from Jackson Laboratories, Bar Harbor, ME.

Mice were housed under a standard 12h:12h Light:Dark cycle and fed *Ad libitum* with normal mouse chow (Harlan 2920X). Food was removed 4–5 hours prior to CO_2 induced narcosis

(method of euthanasia). All procedures were approved and conducted in accordance to the Scripps Florida Institutional Animal Care and Use Committee.

Compound Administration

The formulation of REV-ERB agonists was performed as previously described [17]. SR9009 was formulated in a 10mg/ml solution of 15% cremophor, 85% purified water, and pH'd to 7.0. Injections of 100mg/kg, (volume: 10μl/gram) were performed intraperitoneally (i.p.) for all animal studies.

General Mouse Studies

For acute injections, SR9009 was injected one time at either ZT0 (lights on) or ZT6, corresponding to the middle of the lights on period. ZT0 injections—groups of animals (n = 6) were sacrificed every six hours at ZT0, ZT6, ZT12 and ZT18. ZT6 injections- groups of animals (n = 6) were sacrificed one hour later at ZT7. For chronic studies, mice were administered SR9009 (100mg/kg, twice a day) for 10 consecutive days. Injections occurred at ZT0 (lights on) and ZT12 (lights off) in order to try and maintain exposure of SR9009 over a 24-hour period and be consistent with previously published dosing regimens of chronic SR9009 administration [17]. Animals (N = 5) were sacrificed at ZT6 after last injection. For REV-ERBβ KO mice, groups of animals (n = 5) were sacrificed at ZT6.

RNA isolation and real time PCR

RNA was extracted from brain by homogenizing the tissue in 800 μL of RNA STAT-60, using a rotor tissue homogenizer (OMNI International). Chloroform was added after complete nucleoprotein complex dissociation at a 1:5 chloroform:STAT-60 ratio. Samples were vortexed for 15 minutes, kept at room temperature for 3 minutes and later centrifuged for 15min at 14,000rpm at 4°C. The supernatant was transferred to a new tube and half volume of isopropanol to that of STAT-60 was added. The samples were placed at room temperature for 5 to 10 minutes and centrifuged at 13,000 rpm, at 4°C for 10 min. The remaining pellet was washed twice with 75% ethanol, speed-vacuum-dried, and re-suspended in 20 μL of water. cDNA was synthesized using a qScript cDNA synthesis kit (Quanta BioSciences), using 1μg per sample in thermocyler (BioRad T100). Quantitative RT-PCR was performed using a 7900HT Fast RT-PCR (Applied Biosystems). Primers were designed using Primer3 (primer3.sourceforge.net). Specificity and validation of the primers were determined using an *In silico* PCR software program (genome.ucsd.edu) and melting curve analysis to eliminate the possibility of primer-dimer artifacts and check reaction specificity. Primer sequences for mouse REV-ERBα (*Nr1d1*), PPO (*Hcrt*), OX_1R (*Hcrtr1*), OX_2R (*Hcrtr2*), and Bmal1 (*Arntl*) genes are as follows:

Nr1d1 forward: 5'-ATGCCAATCATGCATCAGGT-3'
Nr1d1 reverse: 5'-CCCATTGCTGTTAGGTTGGT-3'
Hcrt forward: 5'-AACCACGCTGCGGGTATCCT-3'
Hcrt reverse: 5'-CCCTCCCCGGGGTGCTAAAG-3'
Hcrtr1 forward: 5'-GACTCTCAGCTTCATCGCCCT-3'
Hcrtr1 reverse: 5'-ACGCTGCTGCACTCCATGAC-3'
Hcrtr2 forward: 5'-GAGGATTCCCTCTCTCGTCG-3'
Hcrtr2 reverse: 5'-GGTGTAGGTATTCCCTCCACA-3'
Arntl forward: 5'-CAGGCTAGCTTGATAGGACAGA-3'
Arntl reverse: 5'-CCAGTGTAGGGGTGACTGTAAAC-3'

All data was normalized to Cyclophilin B (*Ppib*) based on stability and consistency of expression across all conditions analyzed:

Ppib forward: 5'-GCAAGTTCCATCGTGTCATCAAG-3'
Ppib reverse: 5'-CCATAGATGCTCTTTCCTCCTG-3'

Statistical analysis

All data are expressed as mean ±S.E.M. All statistical analysis was performed using GraphPad Prism6 software. A two-way analysis of variance (ANOVA) was performed to determine significant differences between Time of day x treatment. One-way ANOVA was used to analyze intra-gene differences across several time points. All other analysis was performed using a Student's *t*-test. (N is indicated in the figure legends). Significance was assessed as follows: * $p<0.05$, ** $p< 0.01$, $^{***}p < 0.001$.

Results

In order to determine whether modulation of REV-ERB activity affected expression of PPO (*Hcrt*), OX_1R (*Hcrtr1*), and OX_2R (*Hcrtr2*), we administered SR9009 to mice at ZT0 and collected brain tissue every six hours to monitor gene expression changes (Fig 1A). Fig 1B shows

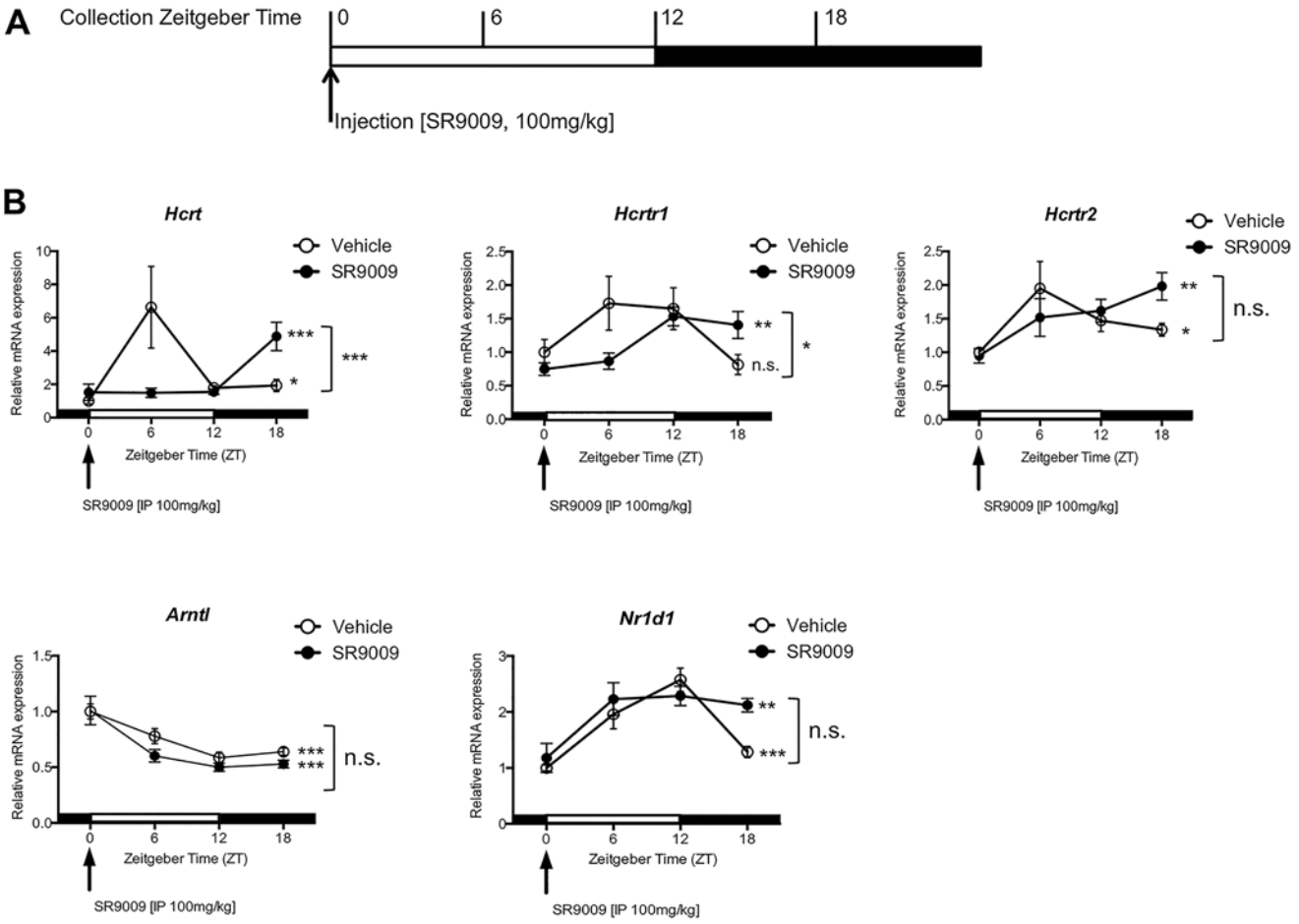

Fig 1. Administration of the REV-ERB agonist, SR9009, at ZT0 affects expression of orexinergic genes in the brain over a 24-hour period. A. Mice were injected at ZT0 with the REV-ERB agonist SR9009 [100mg/kg, i.p.] and tissue was collected every six hours for a twenty-four hour period. The brain was dissected into hypothalamus or reticular formation and processed for mRNA levels at the different time points, ZT0, ZT6, ZT12, and ZT18. **B.** Mice administered with SR9009 or vehicle control were assessed for expression of PPO (*Hcrt)*, Bmal1 (*Arntl*), and REV-ERBα (*Nr1d1)* in the hypothalamus and OX_1R (*Hcrtr1*) and OX_2R (*Hcrtr2*) in the brainstem. N = 6. One way analysis was used to determine intra-gene variation across time. Two-way ANOVA was used to assess differences between groups (time of day x treatment). *$P<0.05$, **$P<0.01$, *and* **$P<0.001$, *n.s. = not significant*.

doi:10.1371/journal.pone.0151014.g001

that, consistent with published data, the mRNA expression of *Hcrt* fluctuates over a 24-hour period, peaking at ZT6 [40]. Surprisingly, SR9009 suppressed this peak in *Hcrt* expression at ZT6 relative to vehicle controls. Furthermore, SR9009 also affected the expression of *Hcrtr1* transcripts, inhibiting the initial upregulation of its expression at ZT6 relative to vehicle control. Similar effects were observed with SR9009 on *Hcrtr2*, although to a lesser degree. REV-ERBα contains a REV-ERB response element (RevRE) in its promoter region and has been shown to modulate its own expression. We also characterized the 24-hour expression pattern of the Bmal1 (*Arntl*) and REV-ERBα (*Nr1d1*) genes after ZT0 injections with SR9009 and observed repression of *Arntl* at ZT6 and little to no change in the expression of *Nr1d1* relative to vehicle control, which is consistent with our previously published data (Fig 1B) [17]. Thus, modulation of REV-ERB activity using a synthetic REV-ERB agonist affects expression of orexinergic genes over a 24-hour period.

We recently demonstrated that acute injections at ZT6, which corresponds to peak REV-ERB mRNA expression, results in increased wakefulness and locomotion as well as reduced rapid-eye movement (REM) and slow-wave sleep (SWS) [51]. Since orexin is implicated in wake and alertness maintenance [33–36, 38, 52–58], we evaluated the effects of acute injections of SR9009 at ZT6 on orexinergic transcripts, as a possible mechanistic pathway mediating REV-ERB agonist wake-inducing effect. Mice were injected at ZT6 and sacrificed at ZT7, the time point where SR9009 has maximal wake-inducing effects, to collect brain tissue (Fig 2A). SR9009 suppressed *Hcrt* and Bmal1 (*Arntl*) in the hypothalamus (Fig 2B and 2C) and *Hcrtr1* and *Hcrtr2* transcript levels in the brainstem of mice (Fig 2D and 2E). *Arntl* was used as a positive control (Fig 2B).

To determine how chronic administration of REV-ERB ligands affects expression of *Hcrtr1* and *Hcrtr2*, we administered SR9009 to mice for 10 days [100mg/kg, i.p., twice a day] after which time mice were sacrificed at ZT6 to collect brains for mRNA analysis. We assessed the expression of orexinergic genes in the hypothalamus, and the brainstem. In the hypothalamus, SR9009 inhibited *Hcrt* expression, with suppression of *Arntl* used as a control. (Fig 3A and 3B). Similarly, in the brainstem, mRNA expression of *Hcrtr1* and *Hcrtr2* were also reduced (Fig 3C and 3D respectively). These results indicate that the REV-ERBs may be repressing orexinergic transcription in these brain areas.

To determine how loss of REV-ERB expression affects orexin gene expression, we used full-body REV-ERB*β* deficient mice to assess *Hcrt* and *Arntl* levels in the hypothalamus, and *Hcrtr1* and *Hcrtr2* levels in the brainstem ([51] and upublished data). Consistent with REV-ERBβ being a transcriptional repressor, we observed increased expression of *Hcrt* and *Arntl* in the hypothalamus (Fig 4A and 4B) and of *Hcrtr1* and *Hcrtr2* in the brainstem (Fig 4C and 4D). These data suggest that REV-ERBβ may act as a transcriptional repressor of *Hcrt*, *Hcrtr1*, and *Hcrtr2*.

Discussion

The REV-ERBs and orexin signaling regulate various aspects of food intake [23, 57, 59, 60], energy expenditure [23, 52, 58, 60–65], and sleep [33, 35, 36, 51, 53–56]. Orexinergic genes are expressed in a circadian manner [40] and the REV-ERBs have been shown to be critical regulators of the circadian rhythm [66]. Given the overlap in functions, we sought to determine whether the REV-ERBs may regulate orexinergic gene expression.

Our results show that modulation of REV-ERB activity affects transcription of the orexinergic genes [PPO (*Hcrt*), OX_1R (*Hcrtr1*), and OX_2R (*Hcrtr2*)]. Acute pharmacological manipulation of REV-ERB activity resulted in alterations of orexinergic genes over a 24-hour period after single injections at ZT0, relative to vehicle control. Similar results were observed with

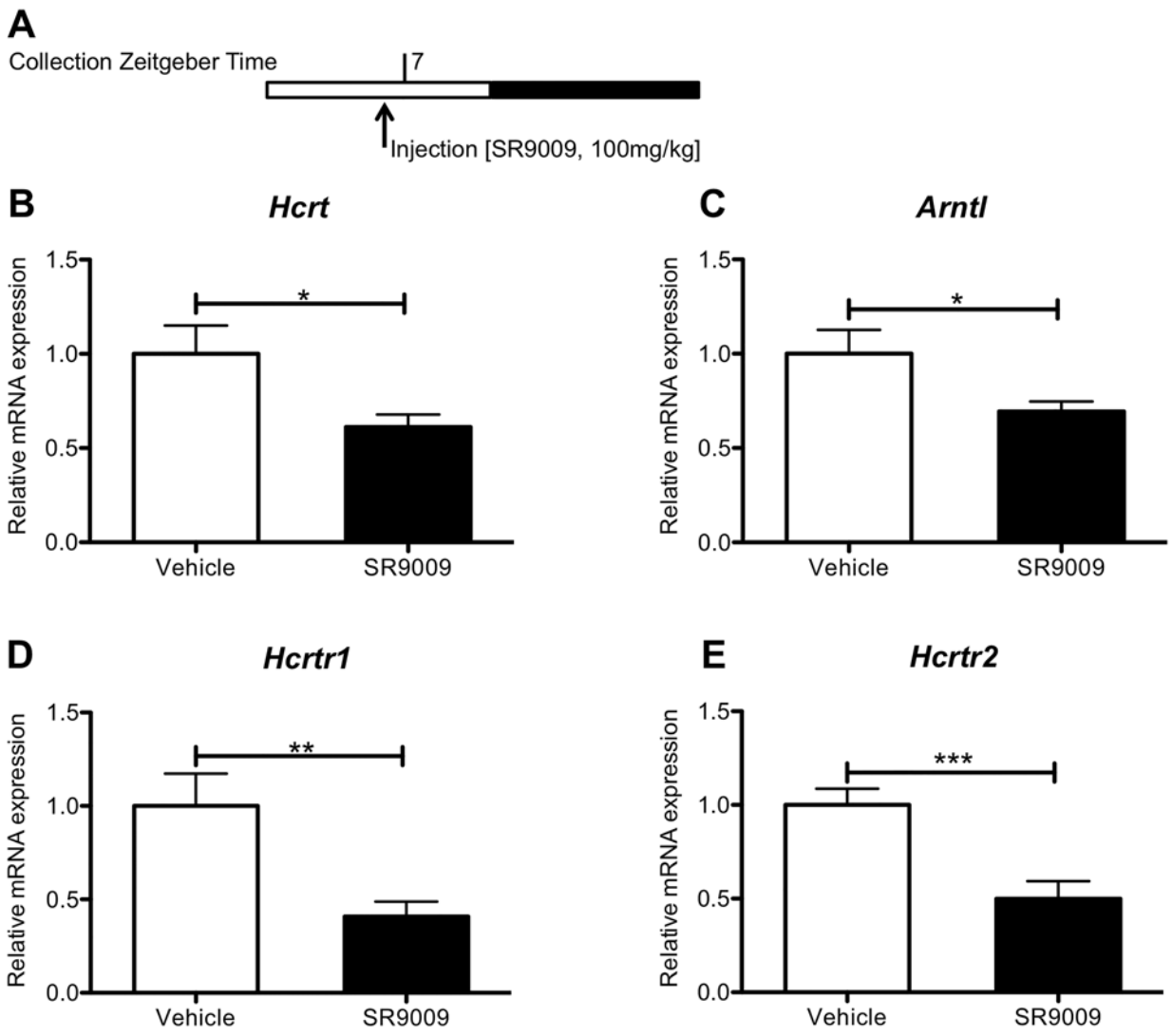

Fig 2. REV-ERB agonist SR9009 administration at ZT6 causes decreased transcript expression of orexinergic genes in the brain. A. Hypothalamic sections were analyzed for transcript levels of PPO (*Hcrt*) at ZT7. **B.** Hypothalamic sections were analyzed for transcript levels of Bmal1 (*Arntl*) at ZT7. **C.** Reticular formation sections were analyzed for transcript levels of OX_1R (*Hcrtr1*) at ZT7. **D.** Reticular formation sections were analyzed for transcript levels of OX_2R (*Hcrtr2*) at ZT7. N = 6. Student's *t*-test was used to assess differences between groups. **P<0.05*, ***P<0.01*, *and ***P<0.001*.

doi:10.1371/journal.pone.0151014.g002

chronic pharmacological manipulation of REV-ERB activity (Fig 3). Delayed/repressed transcription of the orexinergic genes suggests that they may be REV-ERB target genes (Fig 1). Alternatively, orexin is also regulated by peripheral nutrient signaling, directly affecting the hypothalamus [52, 58, 62–65] as well as the nucleus of the solitary tract (NTS) [67]. These signaling macronutrients and peptides may be activated by REV-ERB agonist administration, thus indirectly affecting orexinergic expression. Finally, modulation of orexigeneric gene expression may be due to post-translational effects at the genes, effects incited by nutrient signaling. Future studies examining this phenomenon are warranted in order to definitively determine whether this is the case.

Consistent with the REV-ERBs actively repressing orexinergic genes, mice deficient in REV-ERBβ demonstrate de-repression of orexinergic transcripts at ZT6, which would appear

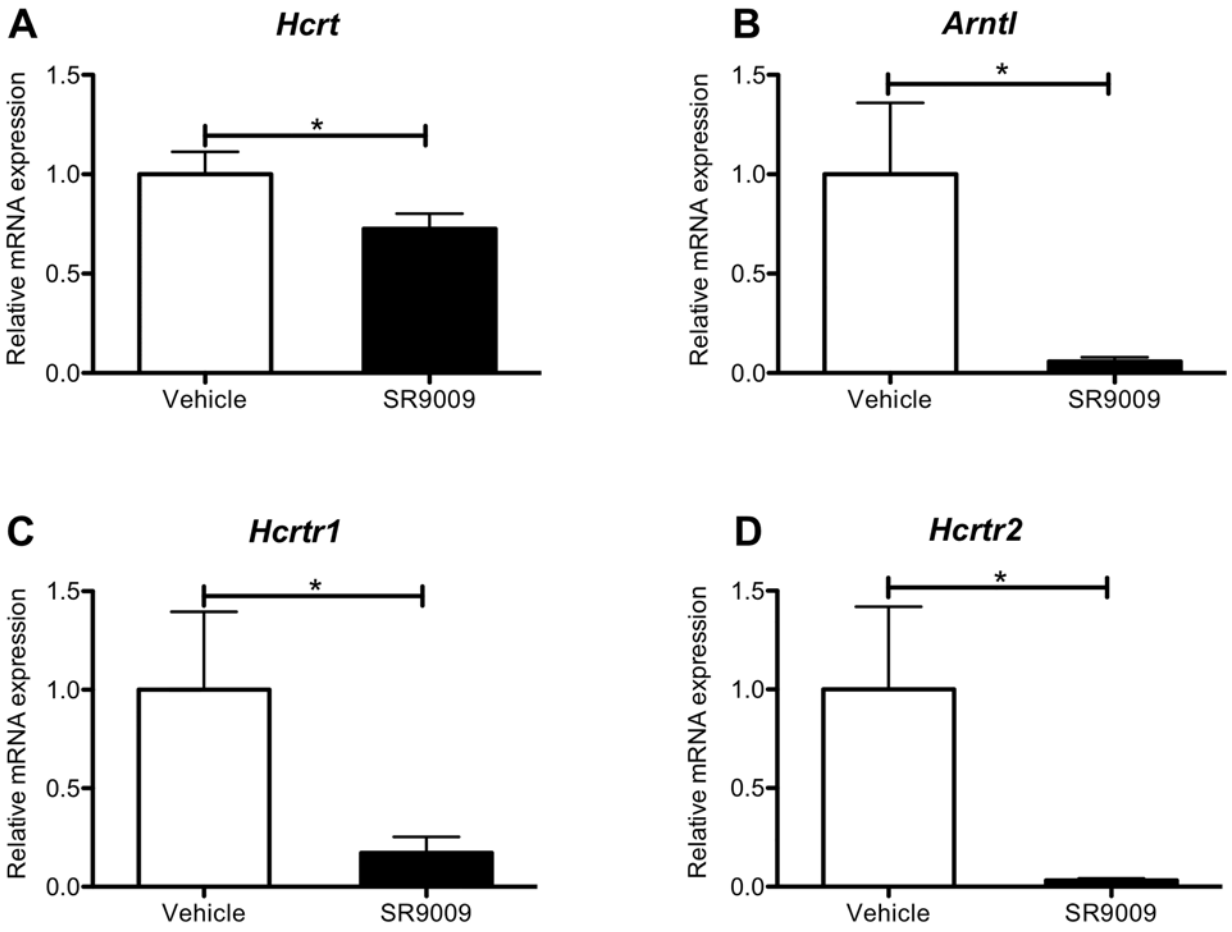

Fig 3. Chronic administration of SR9009 represses orexinergic transcription. Chronic administration of SR9009 results in decreased mRNA levels of **A.** PPO **(***Hcrt)* and **B.** Bmal1 (*Arntl*) in the hypothalamus at ZT6. Chronic administration of SR9009 results in decreased mRNA transcript levels of **C.** OX_1R (*Hcrtr1)* and **D.** OX_2R (*Hcrtr2)* in the brainstem at ZT6. N = 6. Student's *t*-test was used to assess differences between groups. *$P<0.05$, **$P<0.01$, *and* ***$P<0.001$.

doi:10.1371/journal.pone.0151014.g003

to further corroborate direct regulation of *Hcrt*, *Hcrtr1*, and *Hcrtr2* by the REV-ERBs. However, recently published ChIP-seq data generated by the Lazar laboratory determining REV-ERBα binding sites in the brain do not support this as no REV-ERBα binding to any regions in or surrounding orexigenic genes was observed [68]. However, in this data set, REV-ERBα does bind in the promoter region of the Nur77 gene (*Nr4a1*), along with other transcription factors that have been demonstrated to regulate orexin expression, which may account for the changes in orexinergic transcription observed in our studies [69]. REV-ERBα and REV-ERBβ are thought to bind to the same DNA response element and regulate similar genes [18, 70, 71]. In fact, REV-ERBβ is thought to be a redundant protein to REV-ERBβ [18, 71]. Therefore, the ChIP-seq data would suggest that the effects observed with SR9009 treatment and in the absence of REV-ERBβ are indirect effects. However, ChIP-seq studies determining the REV-ERBß cistrome in the brain, coupled with RNA-seq studies are warranted to definitively determine any direct/indirect effects of the REV-ERBs on orexin.

Orexin signaling via OX_1R in the striatum and nucleus accumbens is associated with reward feeding and addictive behavior toward nicotine and other drugs [72–74]. In line with this, our

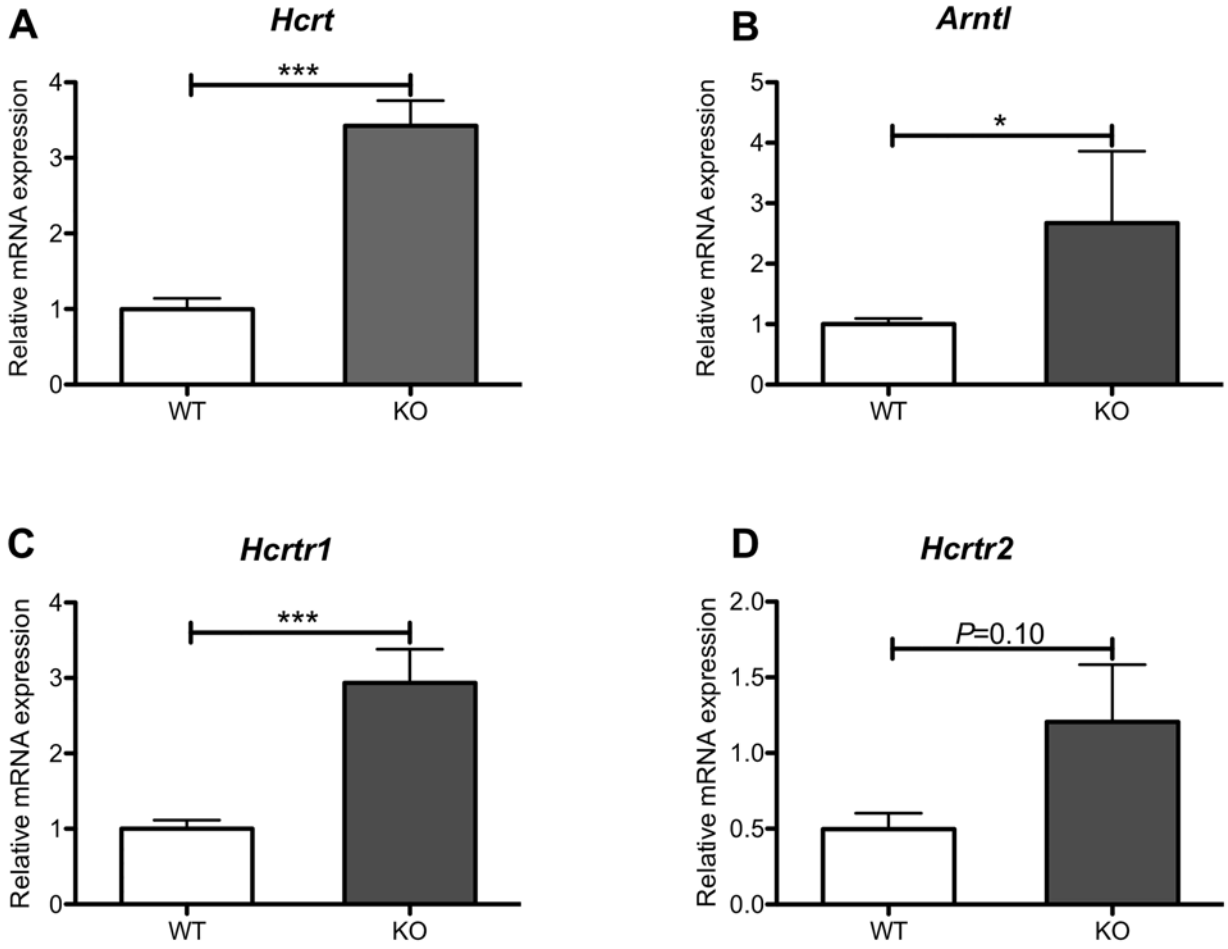

Fig 4. Loss of REV-ERBβ leads to de-repression of orexinergic genes A. PPO (*Hcrt)*, **B**. Bmal1 (*Arntl*) in the hypothalamus, and **C.** OX_1R (*Hcrtr1)* **D.** and OX_2R (*Hcrtr2)* transcript levels in the brainstem were assessed using RT-PCR. Increased orexinergic and *Bmal1* (*Arntl*) transcripts were observed at ZT6 in REV-ERBβ-deficient versus wild-type mice. N = 8. Student's *t*-test was used to assess differences between groups. **P<0.05*, ***P<0.01*, *and* ****P<0.001*.

doi:10.1371/journal.pone.0151014.g004

lab recently demonstrated that administration of SR9009 injection decreased the addictive phenotypic properties of cocaine [19]. Suppression of reward behavior by the REV-ERBs may occur in part via orexinergic signaling. Future mechanistic studies may aid in describing the method of action of the REV-ERBs in reward. Therefore, pharmacological modulation of the REV-ERBs may be a viable therapeutic option to treat addiction, anxiety, and depression via regulation, at least in part, of the orexinergic pathway at the transcriptional level.

Conclusions

Modulation of REV-ERB activity and expression leads to changes in expression of the orexinergic genes *Hcrt*, *Hcrtr1*, and *Hcrtr2*. Our laboratory recently published REV-ERB ligands effects on sleep, anxiety, and metabolism. Given the overlap in REV-ERB pathways with the orexinergic system, our data suggests there may be an interplay between the orexin and REV-ERB signaling pathways. However, further studies exploring this overlap are warranted. Collectively, our data indicate that REV-ERB ligands may be a means to regulate orexin expression and could be a potential therapeutic avenue for disorders associated with aberrant orexin signaling.

Author Contributions

Conceived and designed the experiments: AA LAS TPB. Performed the experiments: AA SB YW. Analyzed the data: AA LAS TPB YW. Contributed reagents/materials/analysis tools: TMK. Wrote the paper: AA LAS TPB.

References

1. Young MW. The molecular control of circadian behavioral rhythms and their entrainment in Drosophila. Annual review of biochemistry. 1998; 67:135–52. Epub 1998/10/06. doi: 10.1146/annurev.biochem.67.1.135 PMID: 9759485.
2. Dunlap JC. Molecular bases for circadian clocks. Cell. 1999; 96(2):271–90. Epub 1999/02/13. PMID: 9988221.
3. Mitsui A, Kumazawa S, Takahashi A, Ikemoto H, Cao S, Arai T. Strategy by Which Nitrogen-Fixing Unicellular Cyanobacteria Grow Photoautotrophically. Nature. 1986; 323(6090):720–2. doi: 10.1038/323720a0 PMID: ISI:A1986E529300058.
4. Stal LJ, Krumbein WE. Nitrogenase Activity in the Non-Heterocystous Cyanobacterium Oscillatoria Sp Grown under Alternating Light-Dark Cycles. Arch Microbiol. 1985; 143(1):67–71. doi: 10.1007/Bf00414770 PMID: ISI:A1985ASH1200013.
5. Aschoff J. Circadian Rhythms in Man—a Self-Sustained Oscillator with an Inherent Frequency Underlies Human 24-Hour Periodicity. Science. 1965; 148(3676):1427–&. doi: 10.1126/Science.148.3676.1427 PMID: ISI:A19656513700009.
6. Aschoff J. Desynchronization and Resynchronization of Human Circadian Rhythms. Aerospace Med. 1969; 40(8):844–&. PMID: ISI:A1969D856400007.
7. Welsh DK, Logothetis DE, Meister M, Reppert SM. Individual Neurons Dissociated from Rat Suprachiasmatic Nucleus Express Independently Phased Circadian Firing Rhythms. Neuron. 1995; 14(4):697–706. PMID: ISI:A1995QU82600004.
8. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, et al. Role of the CLOCK protein in the mammalian circadian mechanism. Science. 1998; 280(5369):1564–9. Epub 1998/06/11. PMID: 9616112.
9. Crumbley C, Burris TP. Direct Regulation of CLOCK Expression by REV-ERB. Plos One. 2011; 6(3). doi: ARTN e17290 doi: 10.1371/journal.pone.0017290 PMID: ISI:000289054600008.
10. Crumbley C, Wang YJ, Kojetin DJ, Burris TP. Characterization of the Core Mammalian Clock Component, NPAS2, as a REV-ERB alpha/ROR alpha Target Gene. J Biol Chem. 2010; 285(46):35386–92. doi: 10.1074/Jbc.M110.129288 PMID: ISI:000283845300016.
11. Guillaumond F, Dardente H, Giguere V, Cermakian N. Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. J Biol Rhythm. 2005; 20(5):391–403. doi: 10.1177/0748730405277232 PMID: ISI:000231824300001.
12. Preitner N, Damiola F, Molina LL, Zakany J, Duboule D, Albrecht U, et al. The orphan nuclear receptor REV-ERB alpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell. 2002; 110(2):251–60. doi: 10.1016/S0092-8674(02)00825-5 PMID: ISI:000177105200012.
13. Bass J, Takahashi JS. Circadian Integration of Metabolism and Energetics. Science. 2010; 330(6009):1349–54. doi: 10.1126/Science.1195027 PMID: ISI:000284902100035.
14. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, et al. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell. 2002; 109(3):307–20. doi: 10.1016/S0092-8674(02)00722-5 PMID: ISI:000175412100007.
15. Ueda HR, Chen WB, Adachi A, Wakamatsu H, Hayashi S, Takasugi T, et al. A transcription factor response element for gene expression during circadian night. Nature. 2002; 418(6897):534–9. doi: 10.1038/Nature00906 PMID: ISI:000177162800041.
16. Hughes ME, DiTacchio L, Hayes KR, Vollmers C, Pulivarthy S, Baggs JE, et al. Harmonics of Circadian Gene Transcription in Mammals. Plos Genet. 2009; 5(4). doi: Artn E1000442 doi: 10.1371/Journal.Pgen.1000442 PMID: ISI:000266320200002.
17. Solt LA, Wang Y, Banerjee S, Hughes T, Kojetin DJ, Lundasen T, et al. Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature. 2012; 485(7396):62–8. Epub 2012/03/31. doi: 10.1038/nature11030 PMID: 22460951; PubMed Central PMCID: PMC3343186.
18. Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, et al. Regulation of circadian behaviour and metabolism by REV-ERB-alpha and REV-ERB-beta. Nature. 2012; 485(7396):123–7. Epub 2012/03/31. doi: 10.1038/nature11048 PMID: 22460952; PubMed Central PMCID: PMC3367514.

19. Banerjee S, Wang YJ, Solt LA, Griffett K, Kazantzis M, Amador A, et al. Pharmacological targeting of the mammalian clock regulates sleep architecture and emotional behaviour. Nat Commun. 2014; 5. doi: Artn 5759 doi: 10.1038/Ncomms6759 PMID: ISI:000347174900001.

20. Jager J, O'Brien WT, Manlove J, Krizman EN, Fang B, Gerhart-Hines Z, et al. Behavioral Changes and Dopaminergic Dysregulation in Mice Lacking the Nuclear Receptor Rev-erb alpha. Mol Endocrinol. 2014; 28(4):490–8. doi: 10.1210/Me.2013-1351 PMID: ISI:000335452100006.

21. Bernardis LL, Bellinger LL. The Lateral Hypothalamic Area Revisited—Neuroanatomy, Body-Weight Regulation, Neuroendocrinology and Metabolism. Neurosci Biobehav R. 1993; 17(2):141–93. PMID: ISI:A1993LC89200002.

22. Bernardis LL, Bellinger LL. The lateral hypothalamic area revisited: Ingestive behavior. Neurosci Biobehav R. 1996; 20(2):189–287. PMID: ISI:A1996UV64800002.

23. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998; 92(5):1 page following 696. Epub 1998/04/04. PMID: 9527442.

24. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in orexin knockout mice: Molecular genetics of sleep regulation. Cell. 1999; 98(4):437–51. PMID: ISI:000082174900005.

25. Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. P Natl Acad Sci USA. 1999; 96(2):748–53. PMID: ISI:000078189200079.

26. De Lecea L, Kilduff TS, Peyron C, Gao XB, Foye PE, Danielson PE, et al. The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. P Natl Acad Sci USA. 1998; 95(1):322–7. PMID: ISI:000071429500062.

27. Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci. 1998; 18(23):9996–10015. PMID: ISI:000077169800038.

28. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior (vol 92, pg 573, 1998). Cell. 1998; 92(5):U29–U. PMID: ISI:000072406000015.

29. Levitt DR, Teitelbaum P. Somnolence, Akinesia, and Sensory Activation of Motivated Behavior in Lateral Hypothalamic Syndrome. P Natl Acad Sci USA. 1975; 72(7):2819–23. PMID: ISI:A1975AM76900075.

30. Danguir J, Nicolaidis S. Cortical Activity and Sleep in the Rat Lateral Hypothalamic Syndrome. Brain Res. 1980; 185(2):305–21. PMID: ISI:A1980JG44400008.

31. Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, et al. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci. 2000; 20 (22):8620–8. PMID: ISI:000165131500049.

32. Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. P Natl Acad Sci USA. 1999; 96(19):10911–6. PMID: ISI:000082574100071.

33. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. 1999; 98(3):365–76. Epub 1999/08/24. PMID: 10458611.

34. Hungs M, Fan J, Lin L, Lin X, Maki RA, Mignot E. Identification and functional analysis of mutations in the hypocretin (orexin) genes of narcoleptic canines. Genome research. 2001; 11(4):531–9. Epub 2001/04/03. doi: 10.1101/gr.161001 PMID: 11282968.

35. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 1999; 98(4):437–51. Epub 1999/09/11. PMID: 10481909.

36. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet. 2000; 355(9197):39–40. Epub 2000/01/01. doi: 10.1016/S0140-6736(99)05582-8 PMID: 10615891.

37. Aston-Jones G, Smith RJ, Sartor GC, Moorman DE, Massi L, Tahsili-Fahadan P, et al. Lateral hypothalamic orexin/hypocretin neurons: A role in reward-seeking and addiction. Brain research. 2010; 1314:74–90. Epub 2009/10/10. doi: 10.1016/j.brainres.2009.09.106 PMID: 19815001; PubMed Central PMCID: PMC2819557.

38. Boutrel B, Cannella N, de Lecea L. The role of hypocretin in driving arousal and goal-oriented behaviors. Brain research. 2010; 1314:103–11. Epub 2009/12/02. doi: 10.1016/j.brainres.2009.11.054 PMID: 19948148; PubMed Central PMCID: PMC4307927.

39. Thompson JL, Borgland SL. A role for hypocretin/orexin in motivation. Behavioural brain research. 2011; 217(2):446–53. Epub 2010/10/06. doi: 10.1016/j.bbr.2010.09.028 PMID: 20920531.

40. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science. 2005; 308(5724):1043–5. Epub 2005/04/23. doi: 10.1126/science.1108750 PMID: 15845877; PubMed Central PMCID: PMC3764501.

41. Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LH, Guan XM. Distribution of orexin receptor mRNA in the rat brain. FEBS letters. 1998; 438(1–2):71–5. Epub 1998/11/20. PMID: 9821961.

42. Sellayah D, Bharaj P, Sikder D. Orexin Is Required for Brown Adipose Tissue Development, Differentiation, and Function. Cell Metab. 2011; 14(4):478–90. doi: 10.1016/J.Cmet.2011.08.010 PMID: ISI:000296223600008.

43. Kukkonen JP. Physiology of the orexinergic/hypocretinergic system: a revisit in 2012. American journal of physiology Cell physiology. 2013; 304(1):C2–32. Epub 2012/10/05. doi: 10.1152/ajpcell.00227.2012 PMID: 23034387.

44. Perna G, Guerriero G, Caldirola D. Emerging drugs for panic disorder. Expert opinion on emerging drugs. 2011; 16(4):631–45. Epub 2011/10/18. doi: 10.1517/14728214.2011.628313 PMID: 21999303.

45. Kuwaki T. Orexinergic modulation of breathing across vigilance states. Respiratory physiology & neurobiology. 2008; 164(1–2):204–12. Epub 2008/05/06. doi: 10.1016/j.resp.2008.03.011 PMID: 18455970.

46. Williams RH, Burdakov D. Hypothalamic orexins/hypocretins as regulators of breathing. Expert reviews in molecular medicine. 2008; 10:e28. Epub 2008/10/03. doi: 10.1017/S1462399408000823 PMID: 18828950.

47. Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. The Journal of neuroscience: the official journal of the Society for Neuroscience. 1998; 18(23):9996–10015. Epub 1998/11/21. PMID: 9822755.

48. Deng BS, Nakamura A, Zhang W, Yanagisawa M, Fukuda Y, Kuwaki T. Contribution of orexin in hypercapnic chemoreflex: evidence from genetic and pharmacological disruption and supplementation studies in mice. Journal of applied physiology. 2007; 103(5):1772–9. Epub 2007/08/25. doi: 10.1152/japplphysiol.00075.2007 PMID: 17717124.

49. Zhang W, Fukuda Y, Kuwaki T. Respiratory and cardiovascular actions of orexin-A in mice. Neuroscience letters. 2005; 385(2):131–6. Epub 2005/06/09. doi: 10.1016/j.neulet.2005.05.032 PMID: 15941620.

50. Annerbrink K, Westberg L, Olsson M, Andersch S, Sjodin I, Holm G, et al. Panic disorder is associated with the Val308Iso polymorphism in the hypocretin receptor gene. Psychiatric genetics. 2011; 21(2):85–9. Epub 2011/06/15. doi: 10.1097/YPG.0b013e328341a3db PMID: 21666548.

51. Banerjee S, Wang Y, Solt LA, Griffett K, Kazantzis M, Amador A, et al. Pharmacological targeting of the mammalian clock regulates sleep architecture and emotional behaviour. Nature communications. 2014; 5:5759. Epub 2014/12/24. doi: 10.1038/ncomms6759 PMID: 25536025; PubMed Central PMCID: PMC4495958.

52. Burdakov D. Electrical signaling in central orexin/hypocretin circuits: tuning arousal and appetite to fit the environment. The Neuroscientist: a review journal bringing neurobiology, neurology and psychiatry. 2004; 10(4):286–91. Epub 2004/07/24. doi: 10.1177/1073858404263597 PMID: 15271256.

53. Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, et al. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron. 2001; 30(2):345–54. Epub 2001/06/08. PMID: 11394998.

54. Nishino S, Ripley B, Overeem S, Nevsimalova S, Lammers GJ, Vankova J, et al. Low cerebrospinal fluid hypocretin (Orexin) and altered energy homeostasis in human narcolepsy. Annals of neurology. 2001; 50(3):381–8. Epub 2001/09/18. PMID: 11558795.

55. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nature medicine. 2000; 6(9):991–7. Epub 2000/09/06. doi: 10.1038/79690 PMID: 10973318.

56. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron. 2000; 27(3):469–74. Epub 2000/10/31. PMID: 11055430.

57. Tsujino N, Sakurai T. Role of orexin in modulating arousal, feeding, and motivation. Frontiers in behavioral neuroscience. 2013; 7:28. Epub 2013/04/26. doi: 10.3389/fnbeh.2013.00028 PMID: 23616752; PubMed Central PMCID: PMC3629303.

58. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron. 2003; 38(5):701–13. Epub 2003/06/12. PMID: 12797956.

59. Wu T, Ni Y, Kato H, Fu Z. Feeding-induced rapid resetting of the hepatic circadian clock is associated with acute induction of Per2 and Dec1 transcription in rats. Chronobiology international. 2010; 27(1):1–18. Epub 2010/03/09. doi: 10.3109/07420520903398625 PMID: 20205554.

60. Yamanaka A, Sakurai T, Katsumoto T, Yanagisawa M, Goto K. Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain research. 1999; 849(1–2):248–52. Epub 1999/12/11. PMID: 10592311.

61. Duez H, Staels B. Rev-erb-alpha: an integrator of circadian rhythms and metabolism. Journal of applied physiology. 2009; 107(6):1972–80. Epub 2009/08/22. doi: 10.1152/japplphysiol.00570.2009 PMID: 19696364; PubMed Central PMCID: PMC2966474.

62. Thorpe AJ, Teske JA, Kotz CM. Orexin A-induced feeding is augmented by caloric challenge. American journal of physiology Regulatory, integrative and comparative physiology. 2005; 289(2):R367–R72. Epub 2005/06/11. doi: 10.1152/ajpregu.00737.2004 PMID: 15947069.

63. Burdakov D, Alexopoulos H. Metabolic state signalling through central hypocretin/orexin neurons. Journal of cellular and molecular medicine. 2005; 9(4):795–803. Epub 2005/12/21. PMID: 16364191.

64. Chang GQ, Karatayev O, Davydova Z, Leibowitz SF. Circulating triglycerides impact on orexigenic peptides and neuronal activity in hypothalamus. Endocrinology. 2004; 145(8):3904–12. Epub 2004/05/01. doi: 10.1210/en.2003-1582 PMID: 15117877.

65. Karnani MM, Apergis-Schoute J, Adamantidis A, Jensen LT, de Lecea L, Fugger L, et al. Activation of central orexin/hypocretin neurons by dietary amino acids. Neuron. 2011; 72(4):616–29. Epub 2011/11/22. doi: 10.1016/j.neuron.2011.08.027 PMID: 22099463.

66. Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, et al. The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell. 2002; 110(2):251–60. Epub 2002/08/02. PMID: 12150932.

67. Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(2):748–53. Epub 1999/01/20. PMID: 9892705; PubMed Central PMCID: PMC15208.

68. Zhang Y, Fang B, Emmett MJ, Damle M, Sun Z, Feng D, et al. GENE REGULATION. Discrete functions of nuclear receptor Rev-erbalpha couple metabolism to the clock. Science. 2015; 348(6242):1488–92. Epub 2015/06/06. doi: 10.1126/science.aab3021 PMID: 26044300; PubMed Central PMCID: PMC4613749.

69. Tanaka S. Transcriptional regulation of the hypocretin/orexin gene. Vitamins and hormones. 2012; 89:75–90. Epub 2012/05/30. doi: 10.1016/B978-0-12-394623-2.00005–6 PMID: 22640609.

70. Bugge A, Feng D, Everett LJ, Briggs ER, Mullican SE, Wang F, et al. Rev-erbalpha and Rev-erbbeta coordinately protect the circadian clock and normal metabolic function. Genes & development. 2012; 26(7):657–67. Epub 2012/04/05. doi: 10.1101/gad.186858.112 PMID: 22474260; PubMed Central PMCID: PMC3323877.

71. Liu AC, Tran HG, Zhang EE, Priest AA, Welsh DK, Kay SA. Redundant function of REV-ERBalpha and beta and non-essential role for Bmal1 cycling in transcriptional regulation of intracellular circadian rhythms. PLoS genetics. 2008; 4(2):e1000023. Epub 2008/05/06. doi: 10.1371/journal.pgen.1000023 PMID: 18454201; PubMed Central PMCID: PMC2265523.

72. Durieux PF, Bearzatto B, Guiducci S, Buch T, Waisman A, Zoli M, et al. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. Nat Neurosci. 2009; 12(4):393–5. doi: 10.1038/Nn.2286 PMID: ISI:000264563100012.

73. Yawata S, Yamaguchi T, Danjo T, Hikida T, Nakanishi S. Pathway-specific control of reward learning and its flexibility via selective dopamine receptors in the nucleus accumbens. P Natl Acad Sci USA. 2012; 109(31):12764–9. doi: 10.1073/Pnas.1210797109 PMID: ISI:000307538200095.

74. Witten IB, Lin SC, Brodsky M, Prakash R, Diester I, Anikeeva P, et al. Cholinergic Interneurons Control Local Circuit Activity and Cocaine Conditioning. Science. 2010; 330(6011):1677–81. doi: 10.1126/Science.1193771 PMID: ISI:000285390500067.