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Light-Dark Cycle Disruption in Hypothalamus Tissue of Mus Musculus Causes Change in the Expression of Gm45928 Gene

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Abstract

The majority of organisms on earth display consistent 24-hour rhythms in their physiology and behavior due to circadian biology. Light and dark cycles play a key role in setting our internal body clock, which controls things like our sleep patterns, hormone levels, body temperature, and metabolism. The disruption of the light-dark cycle has significant effects on the molecular and behavioral rhythms of the circadian clock of hypothalamus. Gm45928 is a locus that represents naturally occurring read through transcription between the neighboring Clcf1 and Pold4 genes on chromosome 19. We employed transcriptomic analysis to investigate the gene expression patterns in hypothalamus tissues of M. musculus in varying light-dark conditions and different diet, antibiotic treatment conditions. To perform the analysis, we utilized the R Studio software, which provided us with a comprehensive set of tools and packages, enabling us to process and analyze the transcriptomic data efficiently. Comparing between the two treated groups, CD and ABX, reveals significantly higher gene expression, suggesting a potential protective effect of ABX treatment against gut microbes compared to the CD group.

Keywords: Immune cell, cellular function, metadata, differential gene expression, normalization, circadian rhythm, fold change, hypothalamus tissue

INTRODUCTION

The majority of organisms on earth display consistent 24-hour rhythms in their physiology and behavior due to circadian biology. These rhythms are driven by the earth's rotation, marked by the rising and setting of the sun. At molecular, cellular, and behavioral levels, circadian biology operates through an internal clock present in almost all cells. The master clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, helps synchronize circadian clocks throughout the body. Additionally, other hypothalamic nuclei play a vital role in regulating physiological rhythms, such as the sleep-wake cycle and daily food intake [1, 2].

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At the core of the circadian clock are transcription factors called CLOCK and BMAL1, which control the expression of output genes, including Per and Cry. These genes are crucial for maintaining the rhythmicity of the clock. The circadian clock also involves feedback loops with NR1D1 and RORs, as well as the metabolite NAD+ and the enzyme SIRT1, all of which contribute to regulating the clock. Knockout of Bmal1 disrupts rhythmicity, emphasizing its essential role in maintaining cellular and behavioral rhythms. The circadian clock's resilience and intricate nature enable it to keep time in normal light-dark cycles, allowing organisms to adapt and synchronize their internal clocks with the external environment [1, 3–5].

The circadian regulation controls the immune system. Immune cell activities, such as cytokine production and response, follow a circadian pattern according to clock genes in immune cells. Disruptions of such rhythms occur via sleeping disorders and/or environmental causes relating to light, temperature, and food intake-all of which disrupt immune functions and point to an interwoven nature between circadian biology and health [2, 6–9].

Light-dark cycles are considered as major cues for the entrainment of the circadian system, regulating physiological processes which include the sleep-wake cycle, hormone secretion, body temperature, and metabolism. A uniform day with light instills wakefulness, but sleep is signaled in darkness, thus keeping the internal clock in synchrony with the external environment. The cause of desynchrony between these two interlinking factors would come about if the normal cycle was disrupted continuously [3, 10, 11].

The chow diets are one of the standards for laboratory mice and balance nutritional components properly. Regarding the studies concerned with the light-dark condition and its effect on mice hypothalamus, chow dieting acts as a control to maintain the intake of diet consistent among the experimental groups. This will help the researchers to get the actual effect of lighting conditions on hypothalamic function without the interference of diet [4, 12–15].

Gm45928, also known as Clcf1-pold4, is a locus that represents naturally occurring read through transcription between the neighboring Clcf1 (cardiotrophin-like cytokine factor 1) and Pold4 (polymerase (DNA-directed), delta 4) genes on chromosome 19. This gene has a ubiquitous expression in spleen adult (RPKM 64.7), mammary gland adult (RPKM 42.7) and 27 other tissues. [5, 16–20].

OBJECTIVE

The objective of this study is to investigate how disrupted light-dark (LD) cycles affect hypothalamic gene expression in Mus musculus, with a specific focus on the Gm45928 gene, a potential regulator of circadian rhythms. By comparing gene expression profiles under various LD conditions and dietary interventions (standard chow diet vs. antibiotic-treated), we aim to identify the molecular consequences of circadian disruption. The study further seeks to understand how downregulation of Gm45928 may contribute to altered sleep-wake behaviors and stress response pathways, providing insights into potential strategies to mitigate the adverse effects of environmental circadian misalignment [21–23].

The study investigated how different light-dark cycles, from 0 hours of light/24 hours of dark to 24 hours of light/0 hours of dark, and diet – antibiotic treatments – such as chow diet group versus antibiotic-treated group-affect differential gene expression in hypothalamic tissues in *Mus musculus*. In these tissues, the differential expression of genes will be visualized by techniques, such as MA plots, heatmaps, volcano plots, UMAP plots, bar plots, and box plots. Transcriptomic analysis in R Studio will look into exploring and interpreting gene expression patterns within the hypothalamus.

MATERIALS AND METHODS

The raw data used in this study was obtained from Zhen Y who conducted a comprehensive experiment of "Comparative transcriptome data of multi tissues in response to different environmental light-dark cycles." We acknowledge their contributions and thank them for sharing the data.

While adhering to the methodology presented by Zhen Y, we made some adjustments to suit the specific requirements of our analysis.

Study Design

In this study, 240 C57BL/6N mice, ten weeks of age, were chosen for inclusion and maintained under uniform laboratory conditions to ensure environmental uniformity and a uniform diet. The animals acclimatized for seven days before randomly being divided into two groups: the chow diet group, CD

(n = 120); and the antibiotics treatment group, ABX (n = 120). Both the ABX and control mice were administered with autoclaved drinking water and pellet feed prepared with corn, soybean, wheat, chicken and fish meal, vegetable oil, and various customized vitamins and trace elements. Mice in the ABX group received the same diet, adding to it broad-spectrum antibiotics given in their drinking water. Thereafter, the 240 mice were randomly divided into sub-groups according to different light-dark cycles: (1) 0 h light/24 h dark with CD (CD_LD0/24, n = 24); (2) 8 h light/16 h dark with CD (CD_LD8/16, n = 24); (3) 12 h light/12 h dark with CD (CD_LD12/12, n = 24, control); (4) 16 h light/8 h dark with CD (CD_LD16/8, n = 24); and (5) 24 h light/0 h dark with CD (CD_LD24/0, n = 24). Animals were singly housed in an environmentally controlled facility where the light-dark cycles were manipulated using LED light strips providing 150–200 lux where conditions were strictly maintained at 22–24°C with humidity between 55–65%. The experiment was conducted over a period of 42 days under these controlled environmental conditions [24–28].

Transcriptomic Analysis using R Studio

Transcriptomic analysis: We have analyzed gene expression patterns in hypothalamus tissues of "*Mus musculus*" exposed to light-dark cycles, diets, and antibiotic treatments. The analysis was performed in R Studio, enabling us to use a sophisticated toolkit that would provide speed in processing and exploring the data [29].

Raw transcriptomic data were imported in R Studio in CSV format, first read into a data frame. Subsequently, metadata was generated to provide information about sample IDs, light-dark cycles, treatment groups, and conditions. Further, differential expression analysis was carried out by using DESeq2. This involved the generation of a DESeqDataSet object by combining count data with metadata and specifying the experimental design, considering light-dark cycles and treatment groups. Pre-filtering was applied to ensure data quality, retaining genes with more than 10 raw counts. After that, size factors were estimated, and data normalization was performed. Data were log-transformed in the downstream analysis using a variance-stabilizing transformation, and log2 fold change was calculated comparing gene expression between groups using 0.05 as the alpha level for significance testing.

To facilitate better visualization of the results from differential expression, various plots were generated. An MA plot showing the distribution of changes in gene expression was made using the ggplot2 package. A heatmap of significant genes was constructed using the libraries pheatmap and RColorBrewer, where hierarchical clustering of rows and columns was performed to seek out patterns in gene expression across light-dark cycles and treatments. First, the volcano plot was developed to display log2 fold change against the –log10 adjusted p-value that defines significant differentially expressed genes. This has been implemented using ggplot2.

UMAP analysis was performed to reduce the dimensionality of the log-transformed data and then visualize sample clustering according to their transcriptional profiles. Visualize the result of UMAP, coloring the points of the samples by experimental group [30, 31].

Expression levels across different groups were finally compared by the generation of box plots for the top 10 differentially expressed genes via the ggplot2 library. For a trend in overall expression across experimental conditions, bar graphs were produced through the iDEP software, further supporting the findings from differential expression. These visualization techniques helped provide a comprehensive view of gene expression dynamics in response to the varied light dark and treatment conditions.

RESULTS

In the initial stage of our analysis, the samples underwent a normalization process. This involved creating metadata, which contains information about each sample, such as sample identifiers, experimental conditions, and other relevant details. The raw data obtained from the transcriptomic analysis was then processed and normalized using established methods to minimize technical variations and ensure comparability between samples (Tables 1 and 2).

Table 1. Raw data.

	HYP_CD_LD024_1	HYP_CD_LD024_2	HYP_CD_LD024_3	HYP_CD_LD816_1	HYP_CD_LD816_2	HYP_CD_LD816_3
Gm24018	0	0	0	2	0	0
Gm35703	0	0	0	0	0	0
D4Ertd617e	19	46	36	11	28	26
E430016F16Rik	0	0	0	0	0	0
Mir3473c	0	3	0	0	0	0
Slc19a1	1042	893	1117	1048	913	1061
Gabre	1239	1975	1562	1504	1109	1935
Tex9	1413	1632	1685	1158	1113	1633
Gm34008	0	0	0	0	0	6
Pgbd5	3337	2687	5644	2937	2717	2989
LOC108168713	0	0	0	3	4	0
Gm12468	0	0	3	0	0	4
Lcor	802	993	929	948	700	924
Traj27	3	2	3	1	3	0
Mc5r	60	38	46	78	59	41
Gm13408	222	213	215	187	165	176
Olfr493	0	0	0	0	0	0
Rps12-ps15	0	0	0	0	0	0

Table 2. Metadata.

Samples	LD_Cycles	Treatment	Group
HYP_CD_LD024_1	LD0/24	CD	CD_LD0/24
HYP_CD_LD024_2	LD0/24	CD	CD_LD0/24
HYP_CD_LD024_3	LD0/24	CD	CD_LD0/24
HYP_CD_LD816_1	LD8/16	CD	CD_LD8/16
HYP_CD_LD816_2	LD8/16	CD	CD_LD8/16
HYP_CD_LD816_3	LD8/16	CD	CD_LD8/16
HYP_CD_LD1212_1	LD12/12	CD	CD_LD12/12
HYP_CD_LD1212_2	LD12/12	CD	CD_LD12/12
HYP_CD_LD1212_3	LD12/12	CD	CD_LD12/12
HYP_CD_LD168_1	LD16/8	CD	CD_LD16/8
HYP_CD_LD168_2	LD16/8	CD	CD_LD16/8
HYP_CD_LD168_3	LD16/8	CD	CD_LD16/8
HYP_CD_LD240_1	LD24/0	CD	CD_LD24/0
HYP_CD_LD240_2	LD24/0	CD	CD_LD24/0
HYP_CD_LD240_3	LD24/0	CD	CD_LD24/0
HYP_ABX_LD024_1	LD0/24	ABX	ABX_LD0/24
HYP_ABX_LD024_2	LD0/24	ABX	ABX_LD0/24
HYP_ABX_LD024_3	LD0/24	ABX	ABX_LD0/24
HYP_ABX_LD816_1	LD8/16	ABX	ABX_LD8/16
HYP_ABX_LD816_2	LD8/16	ABX	ABX_LD8/16
HYP_ABX_LD816_3	LD8/16	ABX	ABX_LD8/16
HYP_ABX_LD1212_1	LD12/12	ABX	ABX_LD12/12
HYP_ABX_LD1212_2	LD12/12	ABX	ABX_LD12/12
HYP_ABX_LD1212_3	LD12/12	ABX	ABX_LD12/12
HYP_ABX_LD168_1	LD16/8	ABX	ABX_LD16/8
HYP_ABX_LD168_2	LD16/8	ABX	ABX_LD16/8
HYP_ABX_LD168_3	LD16/8	ABX	ABX_LD16/8
HYP_ABX_LD240_1	LD24/0	ABX	ABX_LD24/0
HYP_ABX_LD240_2	LD24/0	ABX	ABX_LD24/0
HYP_ABX_LD240_3	LD24/0	ABX	ABX_LD24/0

Light and Dark in CD (Chow Diet)

At first the primary objective of our study was to assess the influence of different light-dark (LD) conditions on hypothalamic gene expression in mice fed a normal chow diet. Transcriptomic analysis was conducted to compare the gene expression profiles between LD0.24 vs LD12.12, LD16.8 vs LD12.12, LD24.0 vs LD12.12, and LD8.16 vs LD12.12 conditions. The obtained results provided comprehensive information regarding gene names, their expression levels, pvalues, log2 fold change values, and base mean values. These findings shed light on the intricate patterns of gene expression within the hypothalamus under distinct LD conditions. Importantly, it was observed that not all genes exhibited statistically significant changes in expression compared to the control (chow diet) group. The precise number of genes expressed in each LD condition was meticulously documented, laying the foundation for further in-depth analyses and facilitating a comprehensive understanding of the hypothalamus' response to diverse light-dark cycles.

HYP_CD_LD0.24 vs HYP_CD_LD12.12

Out of 36,234 genes with non-zero read counts in the comparison of HYP_CD_LD0.24 vs. HYP_CD_LD12.12, only 3 showed significant differential expression with an adjusted p-value < 0.05. None of the genes were upregulated since there is no positive value of Log2FoldChange.

However, three of them were downregulated, including Gm44505 (base Mean: 37.84, Log2FoldChange: -24.85, padj: 0.0032), Evx1 (base Mean: 16.22, Log2FoldChange: –22.90, padj: 0.0081), and Gm45928 (base Mean: 7.06, Log2FoldChange: –22.38, padj: 0.0202). The latter genes expression is thus considerably reduced compared to HYP_CD_LD12.12 under the HYP_CD_LD0.24 condition.

HYP_CD_LD16.8 vs HYP_CD_LD12.12

A comparison of expression between HYP_CD_LD16.8 and HYP_CD_LD12.12 showed that 9 genes had an adjusted p-value less than 0.05. Of these, the upregulated genes were 3 in number, namely, Tph1, Fam83a, and Rbp3. Six genes were downregulated, out of which the following genes belonged to the Hox gene family: Hoxb3, Hoxc4, Hoxb5, and Hoxb2; further, Gm45928 and Oas1h. These findings point to the strong upregulation of genes, such as Tph1 and Fam83a, while the downregulation of various Hox genes may suggest possible regulatory changes in developmental pathways.

HYP_CD_LD24.0 vs HYP_CD_LD12.12

Comparing HYP_CD_LD24.0 to HYP_CD_LD12.12, a total of 3 genes are differentially expressed. Among those, Fam83a was upregulated and Gm45928 and Gm20796 were downregulated. The upregulation of Fam83a upregulation suggests a tendency towards higher expression in association with a particular cellular function. Meanwhile, Gm45928 and

Gm20796 are downregulated, which means these are expressed only lower in the condition HYP_CD_LD24.0 as compared to HYP_CD_LD12.12.

HYP_CD_LD8.16 vs HYP_CD_LD12.12

In the comparison between HYP_CD_LD8.16 and HYP_CD_LD12.12, 3 genes were found to be differentially expressed with an adjusted p-value < 0.05. Fam83a was the only gene upregulated, indicating an increase in its expression under the HYP_CD_LD8.16 condition. In contrast, Smr3a and Gm45928 were downregulated, suggesting a reduction in their expression levels compared to HYP_CD_LD12.12. These findings highlight distinct patterns of gene regulation between the two conditions.

Light and dark in CD vs ABX

In further study, we aimed to compare the effects of light and dark conditions on hypothalamic gene expression in mice by examining samples treated with both chow diet and antibiotics (ABX). We observed that certain genes exhibited higher expression levels compared to the expression levels

observed under light and dark conditions in the chow diet-only group. To identify the most significantly expressed genes, we focused on the top 15 genes from the 4434 significantly expressed genes based on their expression levels, p-values, log2 fold change values, and base mean values. These findings provide valuable insights into the intricate interplay between light-dark cycles and antibiotic treatment in modulating gene expression in the hypothalamus. Moreover, they shed light on potential molecular mechanisms underlying the regulation of circadian rhythms and metabolism in response to these combined influences.

HYP_CD vs HYP_ABX

In total there were 2455 genes upregulated and 1979 downregulated between the treatments by CD and ABX with adjusted p-value < 0.05. The top 15 highly expressed genes included Npw, Gm4847, Socs2, Ssb, Ncl, Zfp935, Hdac2, Psip1, Elavl3, Gm8319 and Smarca2, which were highly upregulated, indicative of their increased expression under the condition of CD. While on the other side, genes, like Crx, Gbp4, Ccnyl1, and Rbm3-ps, were downregulated significantly. This suggests that these genes are expressed at lower levels after ABX treatment. This hence demonstrates a significant variation in gene expression between the two conditions of CD and ABX, where the genes responsible for cellular regulations were upregulated quite significantly, while genes related to other biological mechanisms were substantially repressed.

During the analysis comparing the effects of chow diet (CD) and antibiotic treatment (ABX), we considered different durations, including LD0.24 vs LD12.12, LD16.8 vs LD12.12, LD24.0 vs LD12.12, and LD8.16 vs LD12.12. To visualize the results, several plots were generated.

Together, these plots provide valuable insights into the gene expression changes between CD and ABX conditions at different durations.

MA Plot

The MA plot was constructed to illustrate the relationship between log-fold change (M) and average expression (A) of genes (Figure 1). This plot helps identify differentially expressed genes by pinpointing those that deviate from the central axis, indicating significant changes in gene expression levels between conditions.

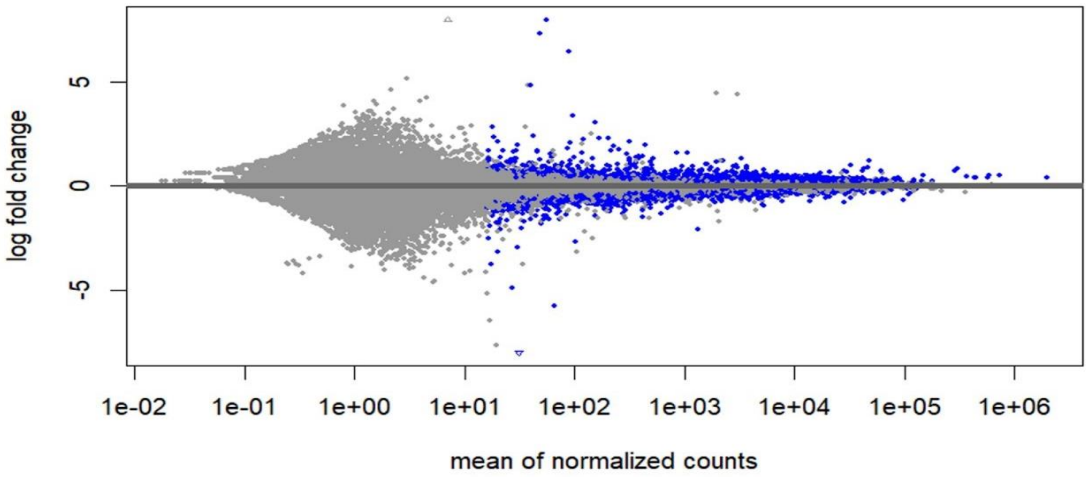

Figure 1. MA plot.

Heat Map

A heat map was created to visualize the overall gene expression patterns across different samples (Figure 2). This graphical representation uses color-coding to depict gene expression levels, allowing for the identification of clusters or groups of genes with similar expression patterns.

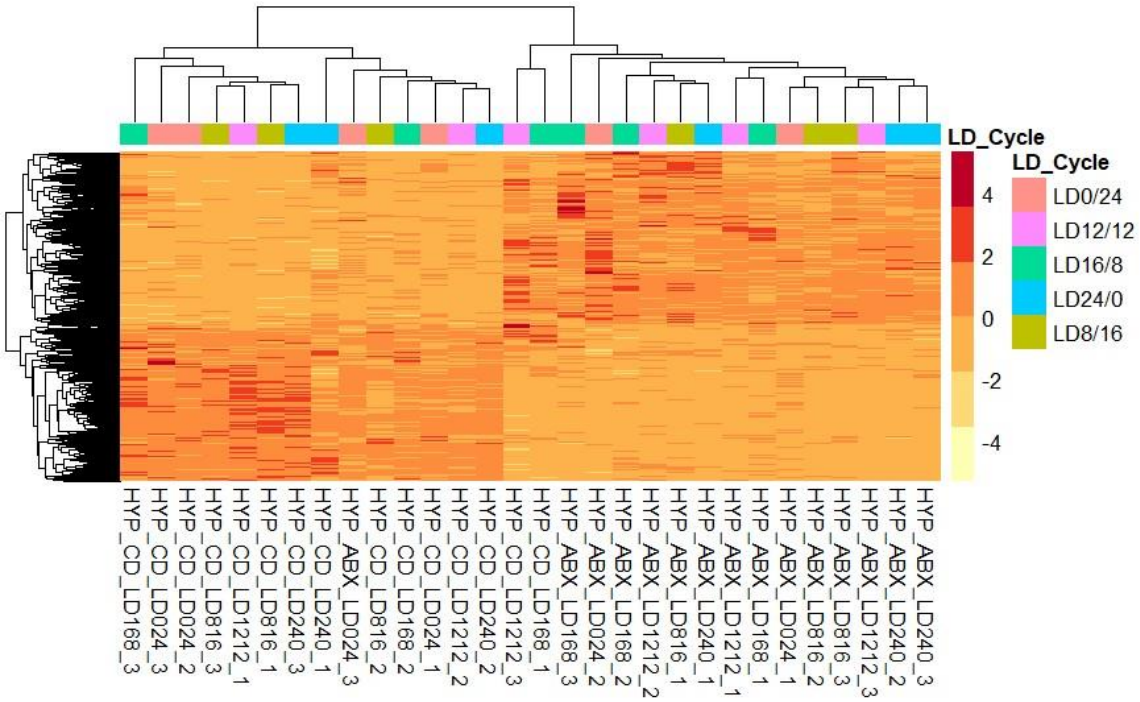

Figure 2. Heat map.

Volcano Plot

Volcano plots were utilized to visualize the statistical significance (p-value) versus the magnitude of gene expression changes (log-fold change) (Figure 3). In these plots, genes with both high statistical significance and large fold changes are represented as points that fall far from the center, forming the "volcano" shape. These genes are considered highly significant and likely to play crucial roles in the observed differences between CD and ABX treatments.

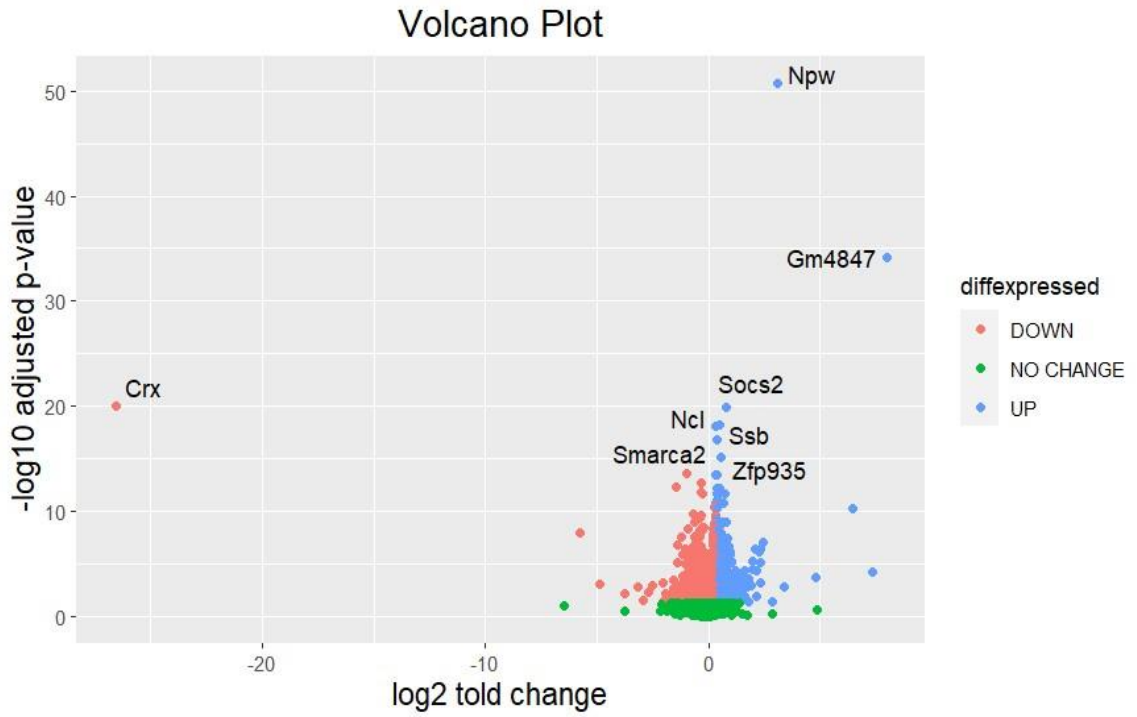

Figure 3. Volcano plot.

UMAP

UMAP (Uniform Manifold Approximation and Projection) was utilized to visualize the transcriptomic data in a lower-dimensional space (Figure 4). UMAP is a dimensionality reduction technique that helps uncover underlying patterns or clusters within the data, enabling a better understanding of the relationship between different samples or conditions.

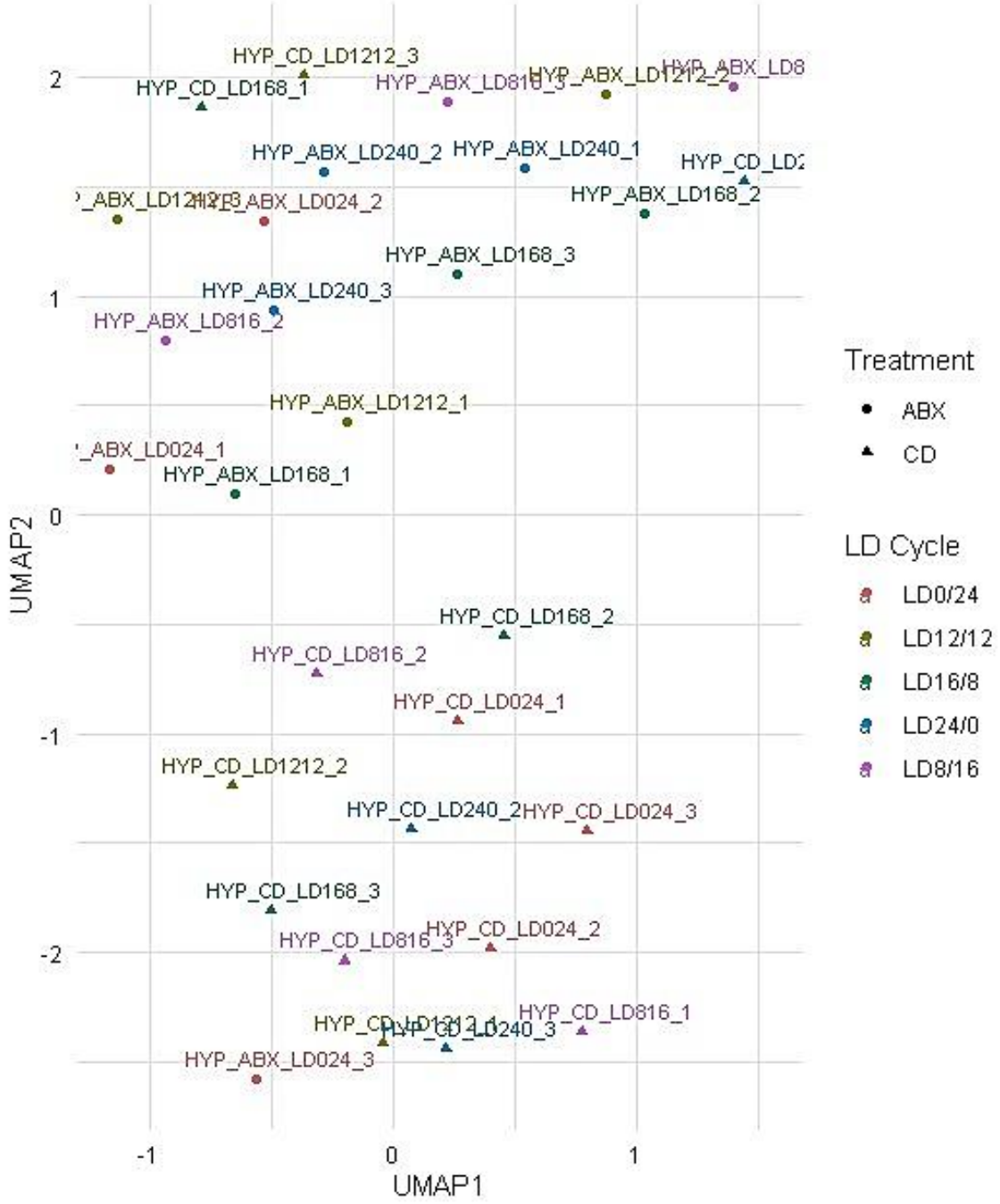

Figure 4. UMAP.

Bar Graph

Bar graphs were created to summarize and compare specific gene expression levels or other relevant data (Figure 5). They provided a concise and visual representation of the quantitative information, facilitating easy interpretation and comparison between CD and ABX treatments.

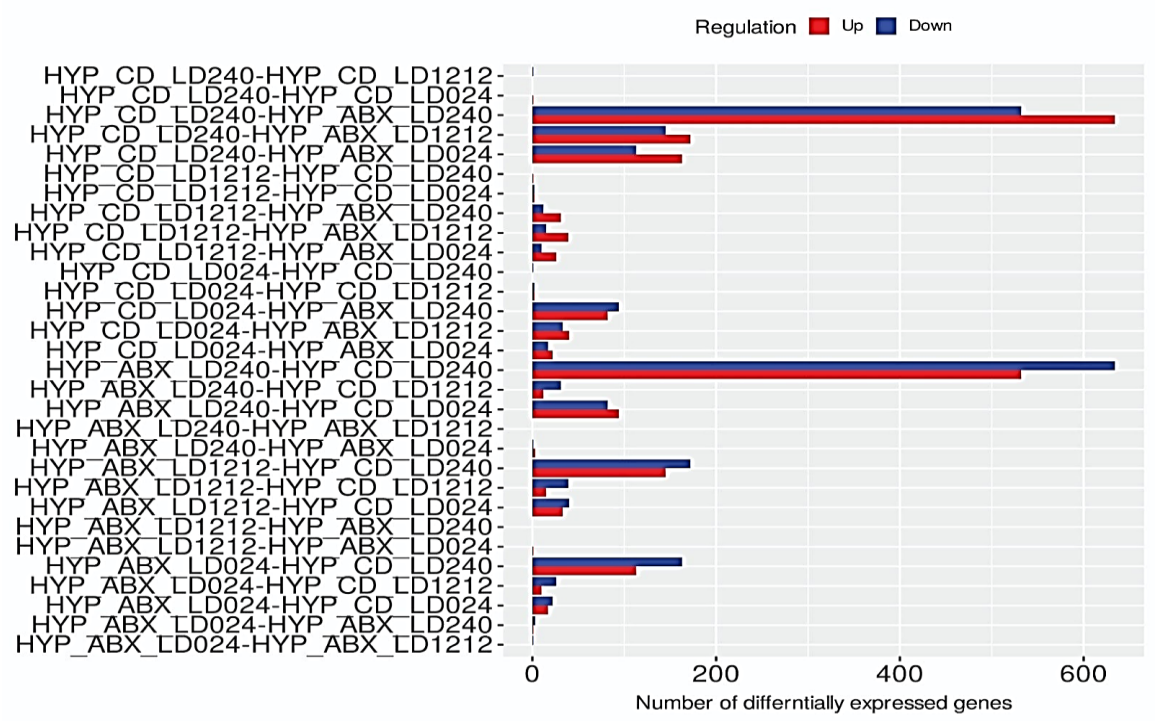

Figure 5. Bar graph.

Box Plot

The box plot analysis was performed on the top 10 genes selected from the 15 significantly expressed genes identified in our study. The box plot provided a visual representation of the distribution of gene expression levels between the chow diet (CD) and antibiotic treatment (ABX) conditions. This plot allowed for a quick assessment of the differences in gene expression distribution between the two treatments. The box plot depicted the median expression level, quartiles, and potential outliers for each gene in CD and ABX conditions (Figures 6–15).

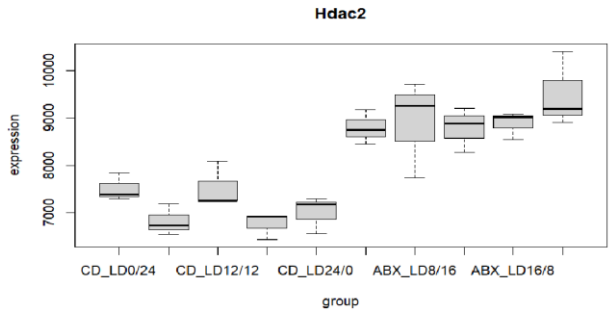

Figure 6. Box Plot for Hdac2.

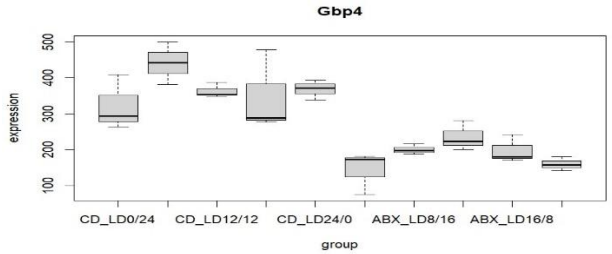

Figure 7. Box Plot for Gbp4.

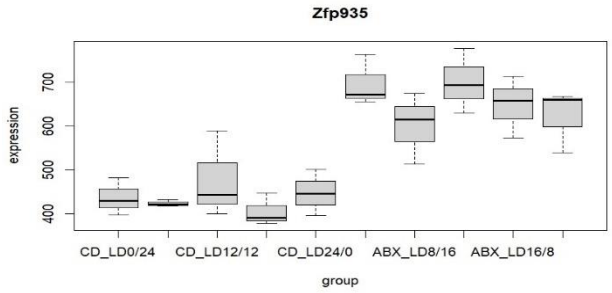

Figure 8. Box Plot for Zfp935.

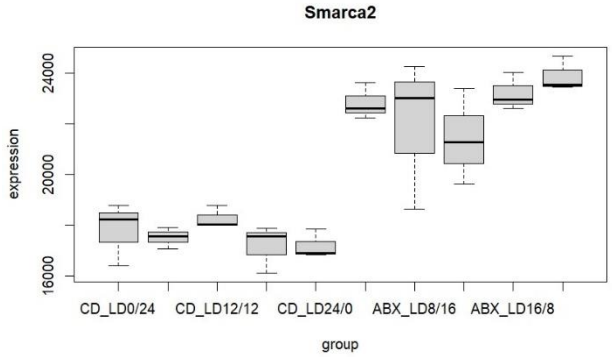

Figure 9. Box Plot for Smarca2.

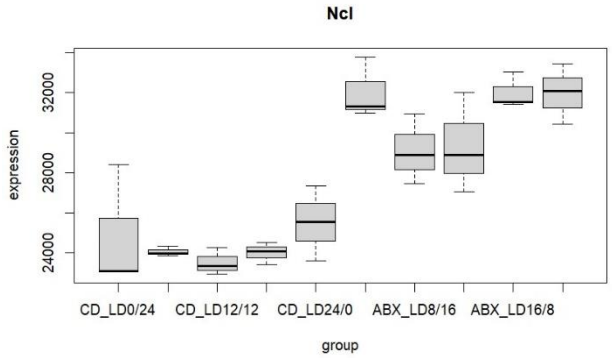

Figure 10. Box plot for Ncl.

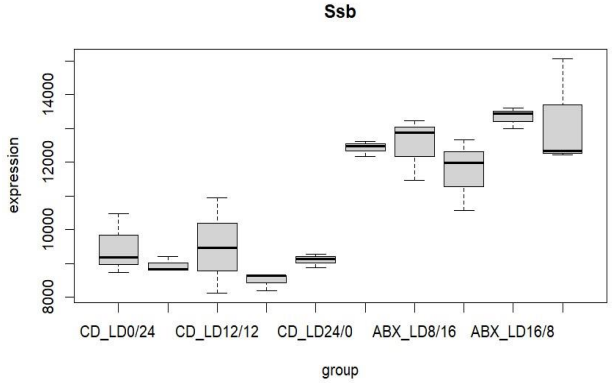

Figure 11. Box plot for Ssb.

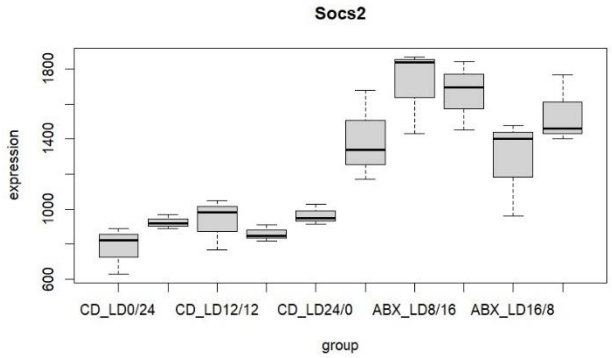

Figure 12. Box Plot for Socs2.

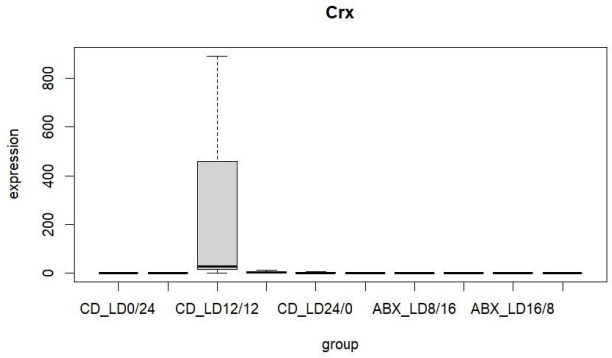

Figure 13. Box Plot for Crx.

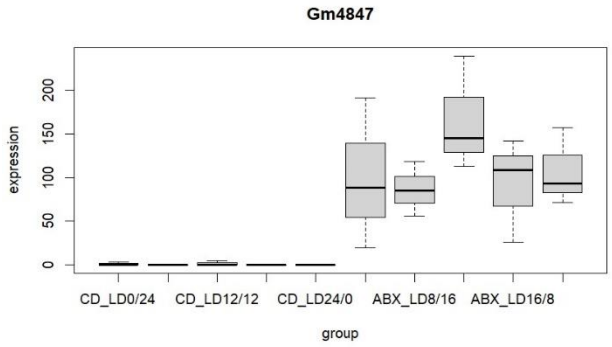

Figure 14. Box plot for Gm4847.

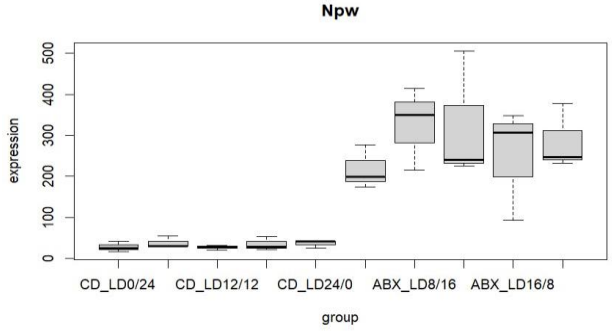

Figure 15. Box plot for Npw.

DISCUSSION

This study examined the impact of varying light-dark (LD) cycle conditions on hypothalamic gene expression in "*Mus musculus*", with a particular focus on the expression of the "Gm45928" gene and its potential role in circadian rhythm regulation. The hypothalamus, a critical regulator of circadian rhythms and immune function, responds to environmental cues, such as LD cycles, cellular signaling, and genetic regulation. Our transcriptomic analysis revealed differential gene expression across several LD conditions, highlighting the complex regulation of gene expression in the hypothalamus.

In our comparison of hypothalamic gene expression across LD conditions (HYP_CD_LD0.24, HYP_CD_LD16.8, HYP_CD_LD24.0, and HYP_CD_LD8.16) with the control LD12.12 condition, we observed limited but significant changes in specific genes. "Gm45928", in particular, was consistently downregulated across several disrupted LD cycles (HYP_CD_LD0.24, HYP_CD_LD16.8, and HYP_CD_LD24.0). This downregulation is noteworthy due to Gm45928's involvement in circadian regulation, where it interacts with core clock components, such as CLOCK and BMAL1 [29, 30].

The Gm45928 gene encodes a protein involved in the regulation of circadian feedback loops through modulation of transcriptional activities mediated by CLOCK and BMAL1, fundamental components of the molecular clock. Reduced expression under disrupted LD cycles would weaken these feedbacks, and the target genes controlled by such circadian regulators would become dysregulated. Thus, sleep-wake behavior modification, metabolic disorders, and impairments of physiological functions controlled by a circadian rhythm could appear [31].

Previous studies have pointed out that Gm45928 controls stress response pathways and neuropeptide synthesis, including vasopressin, involved in circadian rhythmicity. Chronic downregulation of this gene, therefore, as in our study under extreme LD conditions, such as LD0/24 and LD24/0, may contribute to the hypothalamic dysfunction and circadian dysregulation observed. Besides, implication of this gene in stress response mechanisms suggests that chronic downregulation might further negatively affect the consequences of circadian misalignment.

The general small number of differentially expressed genes in our own study would indicate that LD cycle disruption alone may have a more subtle effect on hypothalamic gene expression than perhaps initially thought. However, such downregulation of "Gm45928" is noteworthy and suggests its sensitivity to external environmental cues, such as light exposure, while emphasizing its importance for the integrity of the circadian system [32]. In contrast, the ABX treatment of mice led to more marked changes in gene expression, including upregulation of genes, such as "Npw", "Zfp935", and "Socs2", suggesting that the gut microbiota may modulate hypothalamic gene expression and circadian rhythms. This interaction, which involves the gut microbiota, circadian rhythms, and hypothalamic function, deserves further study because disturbances in these systems might have far-reaching physiological and behavioral implications [33]

Taken together, our results illustrate how diet, light-dark cycle, and hypothalamic gene expression are interconnected. Light-dark conditions had only minimal direct influences on gene expression, but the effects of ABX treatment on gene regulation were significant and point to potential disruptions in physiological and behavioral processes [34]. Such a study brings up the importance of future investigation into the light-dark cycle influence on gene expression within the range of tissues and the involvement of other factors that will modify the circadian rhythm and the immune response. Further studies are needed in the direction of providing a more extensive view of the dynamics of gene regulation and their consequences in health and disease [35–43].

CONCLUSION

The current study exposed a significant effect of disrupted light-dark cycles on the circadian rhythm, sleep pattern, and immune response in the hypothalamus of the species Mus musculus. Disruption in a

light-dark cycle influences molecular and behavioral circadian rhythms; hypothalamic tissues have shown different patterns of gene expression. The transcriptomic analysis, as allowed by the R Studio software, shows that these disrupted light-dark conditions result in a very significant adjustment in gene expression compared to the normal ones. Noticeably, the present study gives evidence for a protective role of antibiotic treatment against the disruption of circadian rhythm regulation when compared with the chow diet group, suggesting an important modulatory role of gut microbes in maintaining stability in circadian rhythm. These results provide greater emphasis on the proper regulation of the light-dark cycle, together with diet and microbiota interaction in maintaining circadian homeostasis. Future studies should further investigate, at the mechanistic level, pathways through which disruptions have an impact on the rhythms, and also investigate possible therapeutic interventions to mitigate such impact caused by disruptions.

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