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Central blockage of sympathetic nerves inhibits the abnormal vital signs and disturbance of the gut microbiota caused by continuous light exposure

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ABSTRACT

Background: Continuous light exposure increases sympathetic excitation in rats, leading to hypertension, left ventricular hypertrophy, and fibrosis. This study was aimed to investigate whether continuous light exposure causes destabilization of vital signs and gut microbiota (GM) in Sprague Dawley (SD) rats and whether clonidine hydrochloride (CH), a central sympathetic depressant drug, could prevent these changes.

Methods: Eight-week-old male SD rats were divided into three groups with different interventions for 14 weeks: control group (CG), 2-mL pure water gavaged daily while on a normal 12-h light/dark cycle; continuous illumination group (CI), 2-mL pure water gavaged daily while receiving continuous exposure to light (300 lx); and drug administration group (DA), CH (10 μg/kg) gavaged daily while receiving continuous exposure to light (300 lx).

Results: The results showed that blood pressure, heart rate, and body weight were significantly higher in the CI group than in the CG and DA groups ($P < 0.05$). Moreover, the Shannon index was higher in the DA group than in the CI group ($P = 0.012$). The beta diversity index in the CG group was significantly higher in the CI group ($P = 0.039$). The pairwise comparison results of the linear discriminant analysis effect size showed that *Oscillospirales* were enriched in the DA group, whereas the *Prevotellaceae* lineage (family level) > *Prevotella* (genus level) > *Prevotellaceae*_bacterium (species level) were enriched in the CI group. The *Muribaculaceae* family was more abundant in the CG group than in the CI group.

Conclusion: Sympathetic nerve inhibition restored the abnormal vital signs and GM changes under continuous light exposure.

1. Introduction

The gut microbiota (GM) is considered a fully-fledged endocrine organ in humans. During the past decade, studies have demonstrated the relationships between GM and several chronic disorders, such as ischemic stroke [1], Parkinson's disease [2], asthma [3],

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type 2 diabetes [4], nonalcoholic fatty liver disease [5], hypertension, and atherosclerosis [6], but the key signatures of the GM have not been established for several of these disorders, including cardiovascular disease (CVD) and even intestinal disorders, such as inflammatory bowel disease [7] and celiac disease [8].

The human circadian system comprises a set of 24-h internal clocks [9], and failed coordination of internal and external processes contributes to circadian rhythm disruption (CRD). Light pollution is ubiquitous in modern society and is a cause of CRD [10]. Increasing evidence has demonstrated that CRD may contribute to CVD pathogenesis [11–16].

Sympathetic nerves (SNs) affect the activity of various organs in the human body. The effects of sympathetic activation on the heart are manifested as increased heart rate (HR), accelerated conduction velocity, increased contractility, and increased ventricular autorhythmicity. Excessive sympathetic activation can lead to high susceptibility to CVD and even recurrent malignant arrhythmias and sudden death. Additionally, elevated sympathetic nervous system activity is a hallmark of hypertension in animals and humans [12] and is one of the causes of refractory hypertension. Sympathetic nerve inhibitors are commonly used to treat tachyarrhythmias, hypertension, and heart failure. Furthermore, previous studies found that continuous light exposure excited the SN [17–19], and excessive sympathetic activation could lead to a high incidence of CVD and even the recurrence of malignant arrhythmias and sudden death. Jing et al. showed that continuous light exposure enhanced sympathetic activity in normal rats, leading to a decreased cardiac function and further sympathetic enhancement in rats with heart failure [13].

Recent studies have suggested a bidirectional interaction between GM and CRD. Deaver et al. have shown that CRD leads to changes in the composition of the intestinal bacterial community and severe dysfunction of the intestinal barrier in mice [17]. GM diurnal rhythmicity also influences the host's circadian activity [18]. Moreover, there is a bidirectional interaction between the GM, brain, and gut [20], with the GM influencing the structure, function and development of the brain in a bottom-up manner via neuroimmune and neuroendocrine mechanisms [21–23]. Numerous studies have shown that GM plays a key role in neurodegenerative diseases. They can synthesize or mimic various neurotransmitters [24]. Dysfunctional microglia participate in the occurrence and development of diseases by releasing a series of pro-inflammatory and neurotoxic factors [25]. The autonomic nervous system of the brain indirectly affects the GM through its ability to regulate intestinal barrier integrity, gastrointestinal motility, secretory processes, and mucosal immune responses [20]. However, no study has clarified the direct influence of SN excitation on changes in the distribution and composition of GM. Therefore, in the present study, we subjected one group of rats to continuous light (CI group) to observe changes in vital signs and GM. We administered a sympathetic inhibitor to another group of rats (DA group) under continuous light to verify whether these changes caused by continuous light could be reversed by sympathetic inhibition.

2. Materials and methods

2.1. Animal procedures and protocols

Fifty-nine healthy clean-grade male (to exclude the effect of estrogen on the GM, male rats were used [26]) Sprague Dawley (SD) rats (188 ± 13 g) were provided by Beijing Huafukang Biotechnology Co., Ltd., Laboratory (Animal Certificate of Conformity No. 1992, License No. SCXK (Shaanxi) 2018-001). The experiments were conducted at the Research Center for High Altitude Medicine. The experiments were approved by the Ethics Committee of the Affiliated Hospital of Qinghai University (approval number: P-SL-2022-050; approval date: 2022-07-01). The animals received humane care in compliance with the guidelines of the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). All animals were housed in individually ventilated rooms at a temperature of 24–26 °C and humidity of 50%–60 %, rats were fed a rat maintenance diet (Beijing Keo Cooperative Feed Co., Ltd.), and food and water were available ad libitum in cages.

Two different lighting conditions were used in this study. The rats were placed either under alternating light and dark conditions for 12 h or under constant light. For alternating light and darkness for 12 h, lighting was controlled using an automatic electrical switch. The light was on from 8 a.m. to 8 p.m. and off from 8 p.m. to 8 a.m. Constant light exposure involved constant LED light with an intensity of approximately 300 lx [27].

The rats were grouped as follows: 1. Control group (CG): 2 mL of pure water gavaged daily and a normal 12-h light/dark cycle; 2. Continuous illumination group (CI): 2 mL of pure water was gavaged daily and continuously exposed to light (300 lx); and 3. Drug administration group (DA): clonidine hydrochloride (CH, 10 μg/kg) was administered daily, and the mice were continuously exposed to light (300 lx).

2.2. Measurement of blood pressure and HR

A Softron BP-2010 (Beijing Softron Biotechnology Co., Ltd. Address: Beijing, China), a noninvasive rat-tail sphygmomanometer was used, and all rats were acclimated to the noninvasive rat-tail sphygmomanometer for blood pressure (BP) and HR measurements for one week. BP and HR were then measured weekly at the same time point using a noninvasive tail-sleeve arterial BP test. The rats were restrained in specific holders and artificially heated to maintain a normal BP. The pulse transducer was fixed and turned on when the participant was quiet. The test started after the pulse wave appeared and stabilized at an interval of 2 min between each test. Each rat was measured three times, and the average value was calculated.

2.3. Rat weight and sample collection

The body weight (BW) of the rats was monitored using an electronic scale (Shanghai Precision Scientific Instrument Co., Ltd. Address: Shanghai, China) weekly after acclimatization. At the end of the experiment, rat feces were collected in sterile tubes within 2

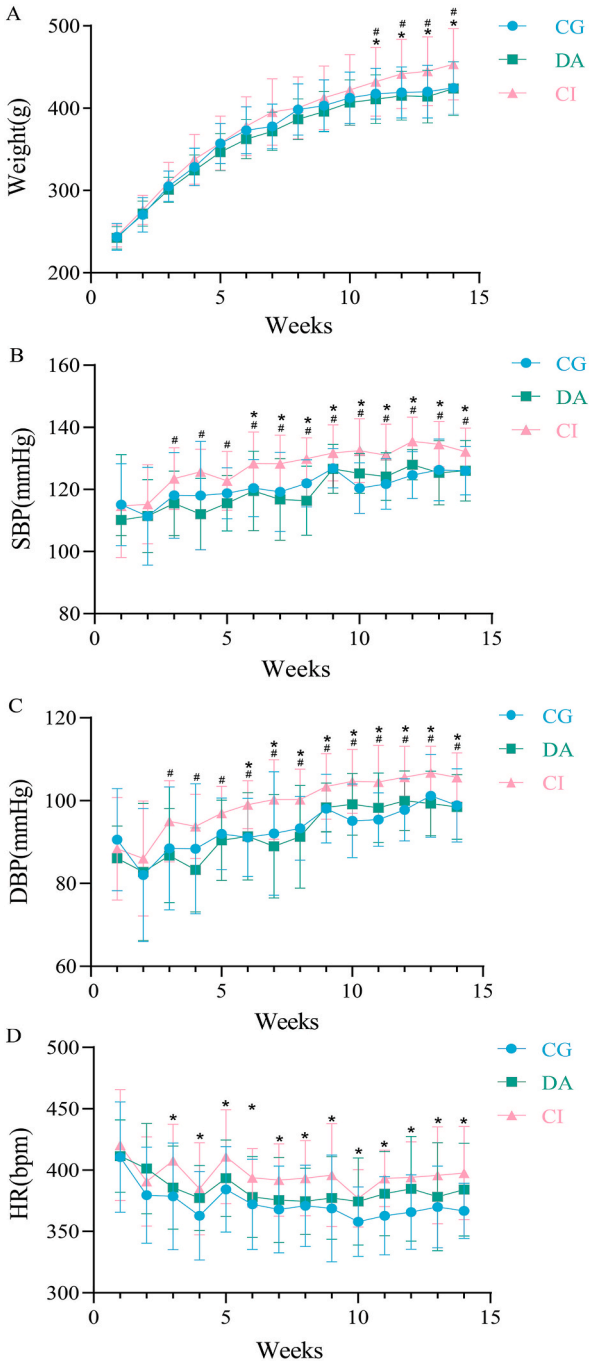

Fig. 1. Effects of continuous light exposure and central sympathetic nerve blocking agents on the body weight (BW), blood presure (BP), and heart rate (HR) of rats. The specific values in the figure are in Supplementary Tables 1 and 2 (A) BW, (B) systolic blood pressure (SBP), (C) diastolic blood pressure (DBP), and (D) HR in control (CG, blue circles), continuous illumination (CI, pink triangles), and drug administration (DA, green squares) rats. BW, SBP, DBP and HR were continuously recorded every 1 week for the study duration. BW of rats in each group was measured by electronic scale; SBP, DBP and HR of rats in each group were measured by using a noninvasive rat-tail sphygmomanometer. # indicates a significant difference between the CI group and the DA group, and * indicates a significant difference between the CI group and the CG group.

h, and the fecal material was stored at −80 °C for 16S rDNA sequencing.

2.4. 16S rDNA sequencing to determine the fecal microbiota of each group of rats

The cetyl trimethyl ammonium bromide (CTAB)/sodium dodecyl sulfate (SDS) method was used to extract total genomic DNA from the samples. The primers used were 341F 5′-CCTAYGGGRBGCASCAG-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′, and Polymerase Chain Reaction (PCR) (98 °C for 1 min; then 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 30 s; and finally at 72 °C for 5 min) was performed to amplify the V3–V4 region of the 16S rDNA gene. Equal volumes of 1 × loading buffer were mixed with the PCR products and electrophoresed on a 2 % agarose gel for DNA detection. The PCR products were mixed in equal proportions, and the mixed PCR products were then purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the NEBNext® Ultra™ IIDNA Library Prep Kit (item number E7645) according to the manufacturer's recommendations. Library quality was assessed on a Qubit@ 2.0 fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on the Illumina NovaSeq platform and 250-bp double-end reads were generated.

Raw data for each sample were first obtained by splitting according to barcodes and removing barcodes and primers, followed by splicing the of R1 and R2 sequence data using FLASH software. The raw data were quality filtered using FASTQ (version 0.20.0) software to obtain high-quality validated data. Each deduplicated sequence generated after noise reduction using DADA2 is called an amplicon sequence variant (ASV) or feature sequence (corresponding to OTU representative sequences). Alpha diversity was calculated based on the seven indices in QIIME2, and beta diversity was calculated based on the weighted and unweighted UniFrac distances in QIIME2. Linear discriminant analysis (LDA) effect sizes (LEfSe) were used to identify categorical biomarkers, with a logarithmic scoring threshold of 4 for LDA to characterize the differences between groups.

2.5. Statistical analysis

Statistical analyses of BP, HR, BW, Chao1, and Shannon index data were performed using IBM SPSS (version 26.0; IBM Ltd., USA). Measurements that conformed to a normal distribution were statistically analyzed using the mean ± standard deviation. BP, HR, and BW were compared among the groups using two-way repeated-measures ANOVA. Differences in the Chao1 index and Shannon indice were assessed using a one-way ANOVA for multiple groups. Post-hoc tests were performed using the least significant difference (LSD) test. To study the significance of differences in community structure between the groups, the Wilcoxon test was performed for beta values using the R package. Taxonomic discovery based on genera was performed using the R package DESeq2. To explore the functional relationship between GM and vital parameters in SD rats, we formulated a correlation matrix based on Spearman's correlation coefficient. Spearman's correlation was calculated and plotted using the R package. The hypothesis test was set as statistically significant at $P < 0.05$. The raw data were sorted, filtered, and assessed for quality to remove chimeras, and ASVs were then generated by noise reduction. After sequencing, species annotation was performed using ASV clustering, and the representative sequences of each ASV were annotated to obtain the corresponding species information and abundance distribution. Fecal gene sequencing was performed by Beijing NoHo Zhiyuan Technology Co.

3. Results

3.1. Changes in BW, HR and BP

BW, BP, and HR are key body parameters that correlate with CVD. Therefore, we first analyzed the changes in these important parameters in different groups of rats.

The BWs of the rats in the CI, CG, and DA groups were measured continuously for 14 weeks. The BW gain of rats in the CI group (11th: 442 ± 42 g, 12th: 445 ± 42 g, 13th: 454 ± 43 g, 14th: 461 ± 44 g) was higher than that in DA group (11th: 415 ± 30 g, 12th: 414 ± 32 g, 13th: 424 ± 33 g, 14th: 429 ± 33 g) and CG group (11th: 419 ± 31 g, 12th: 420 ± 32 g, 13th: 425 ± 32 g, 14th: 429 ± 33 g), showing a significant difference at the 11th, 12th, 13th and 14th weeks of the experiment (11th: CI vs. CG $P = 0.047$, CI vs. DA $P = 0.019$; 12th: CI vs. CG $P = 0.035$, CI vs. DA $P = 0.009$; 13th: CI vs. CG $P = 0.016$, CI vs. DA $P = 0.013$; 14th: CI vs. CG $P = 0.01$, CI vs. DA $P = 0.008$), while there was no significant difference in BW between rats in DA group and CG group ($P > 0.05$) (Fig. 1A).

The BP and HR of rats in the three groups were measured continuously for 14 weeks. Baseline BP and HR also showed no significant differences among the three groups (Fig. 1B–D and Supplementary Tables 1 and 2). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of rats in the CI group were significantly higher than those in the DA and CG groups. From the 3rd week of the experiment, there was a significant difference between the CI group and DA group (3rd: SBP: $P = 0.031$; DBP: $P = 0.034$; 4th: SBP: $P = 0.001$; DBP: $P = 0.006$; 5th: SBP: $P = 0.013$; DBP: $P = 0.017$). From the 6th week of the experiment, there was a significant difference between the CI group and CG group ($P < 0.05$), and these differences lasted until the 14th week. There was no significant difference in BP between the DA and CG groups ($P > 0.05$). (Fig. 1B and C and Supplementary Table 1). From the 3rd week, the HR of rats in the CG group was significantly lower than that of rats in the CI groupand remained so until the 14th week (3rd , $P = 0.014$; 4th, $P = 0.047$; 5th, $P = 0.021$; 6th, $P = 0.037$; 7th, $P = 0.029$; 8th, $P = 0.025$; 9th, $P = 0.039$; 10th, $P = 0.049$; 11th, $P = 0.002$; 12th, $P = 0.013$; 13th, $P = 0.045$; 14th, $P = 0.006$). However, the HR of the rats showed no significant difference between the CI and DA groups or a significant difference between the CG and DA groups (Figure and Supplementary Tables 1 and 2).

These results indicate that the stability of BW, SBP, DBP, and HR is influenced by continuous light exposure, while central sympathetic blockage can reduce the abnormalities in BW, SBP, DBP, and HR caused by continuous light exposure.

3.2. GM alpha and beta diversity in the CI, CG and DA groups

To describe the composition of the GM, 3,458,585 valid datasets were generated from 59 samples, providing 7113 characteristic sequences (ASVs). Notably, 1268 unique ASVs were found in the CG group, 1269 in the DA group, and 1024 in the CI group (Fig. 2A). Shannon dilution curves based on ASVs from all samples reached a plateau, indicating that the amount of sequencing data was sufficiently large to reflect most species information in the samples. To assess the effect of CL on the abundance and diversity of GM, we compared the richness index (Chao1) and diversity index (Shannon) for each group. The Chao1 index indicated that CL did not affect the species abundance in the rat GM (CI vs. DA, P = 0.44; CI vs. CG, P = 0.92; CG vs. DA, P = 0.50) (Fig. 2B). The Shannon index of the rat GM was lower in the CI group in the DA group (CI vs. DA, P = 0.02; CI vs. CG, P = 0.30; CG vs. DA, P = 0.19) (Fig. 2C). This result indicates that the inhibition of sympathetic excitation increases the diversity of the microbiota in the rat gut. Beta diversity (unweighted UniFrac) analysis was performed to detect differences in the microbial composition among all groups (Fig. 2D and E). The

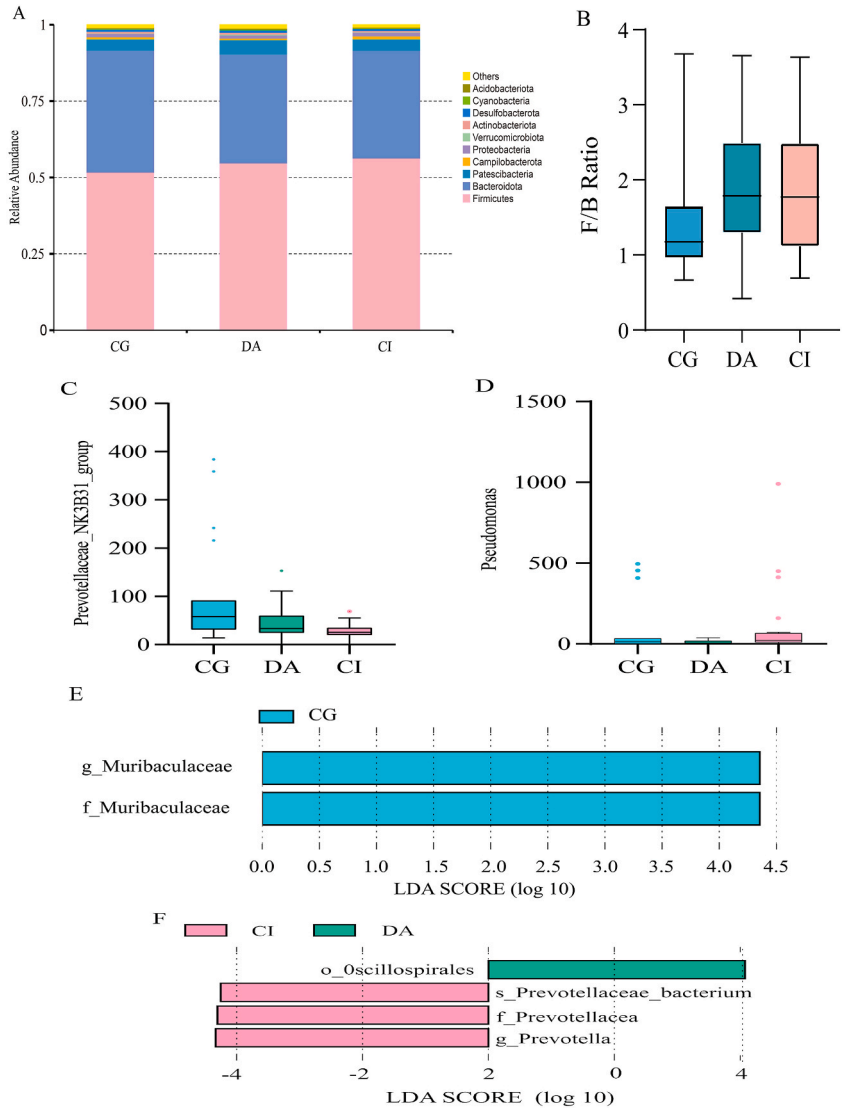

Fig. 2. Altered bacterial microbiota biodiversity and composition in the various groups. (A) Venn diagram shows the common and different gut microbiota of the three groups. (B and C) Chao1 and Shannon indices describing the alpha diversity of the bacterial microbiota in the various groups studied (one-way ANOVA test with the least significance difference (LSD) test). (D) Beta diversity measurements, as indicated by unweighted UniFrac distances. Groups were compared using the Wilcoxon test. Different colors represent different groups of samples. The short horizontal lines of the box from top to bottom represent the 75th, 50th, and 25th percentiles, respectively. (E) Beta diversity index unweighted UniFrac distance matrix heatmap. The larger the circle, the darker the corresponding color, and the greater the difference between the two samples; conversely, the smaller the circle, the lighter the corresponding color, and the smaller the difference between the two samples. *indicates significance at $P < 0.05$. **indicates significance at $P < 0.01$.

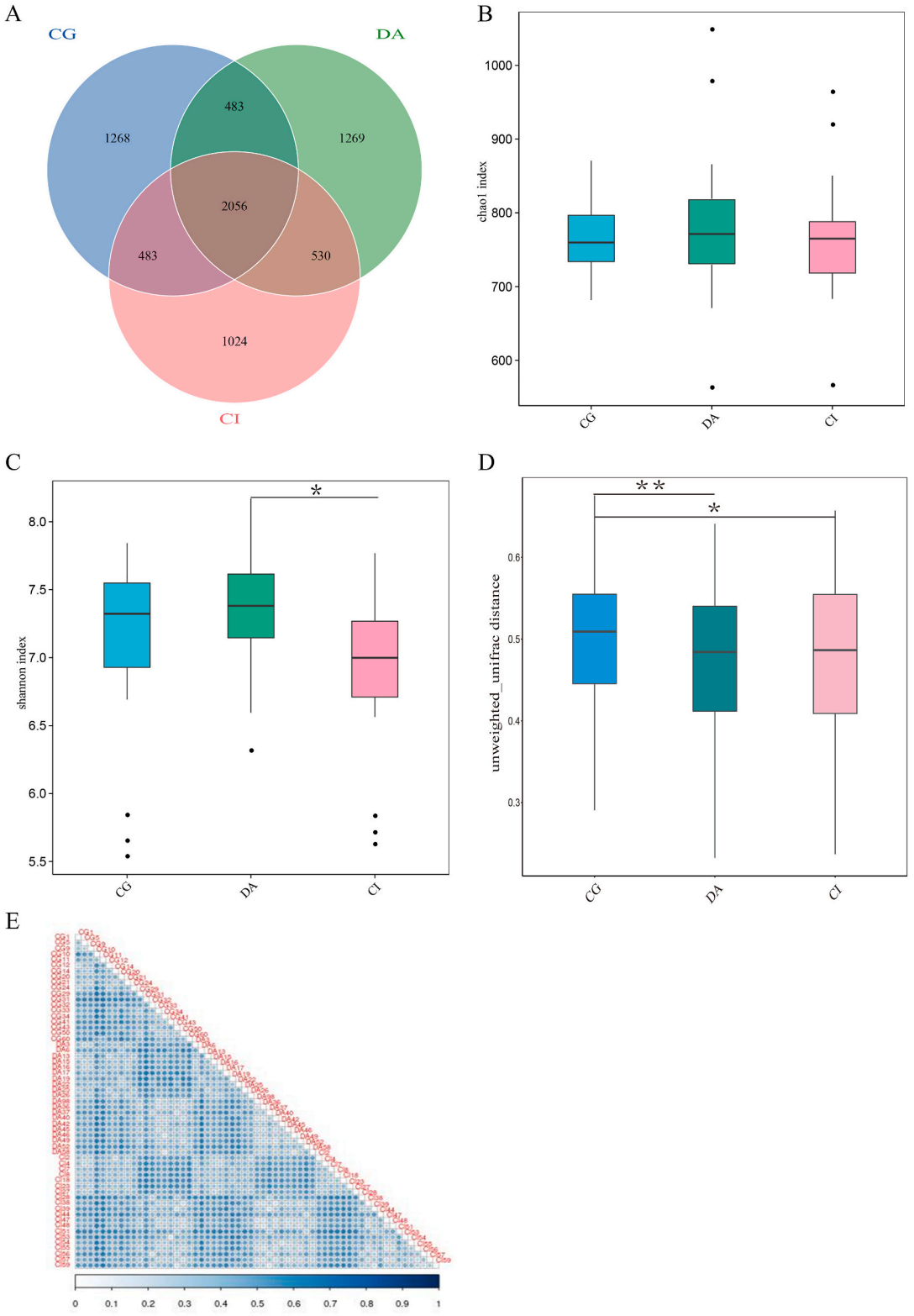

Fig. 3. Different compositions of the gut microbiota (GM) in the control group (CG), drug administration group (DA) and continuous illumination (CI) groups. (A) Relative abundances of the GM at the phylum level in the CG, DA and CI groups. (B) The *Firmicutes/Bacteroidetes* ratio of GM in the CG, DA and CI groups. (C) Calculation of the difference in colony composition between the CG and CI groups based on the DESeq2 negative binomial algorithm. (D) Calculation of the difference in colony composition between the DA and CI groups based on the DESeq2 negative binomial algorithm.

Potential biomarkers, as determined by LEfSe analysis. LEfSe identified significantly different bacterial taxa enriched in each cohort at LDA score >4, P < 0.05, (E) CG vs. CI comparison; (F) DA vs. CI comparison. The prefixes of p, c, o, f, g, and s before the species represent six different taxonomic levels: phylum, class, order, family, genus, and species. Bars represent bacterial species. LEfSe, Linear discriminant analysis effects size.

beta diversity index in the CG group was significantly higher than the DA and CI groups (CG vs. DA, P = 0.005; CG vs. CI, P = 0.039). The analyses mentioned above demonstrated that both continuous light and CH administration caused changes in the microbial composition.

3.3. Different compositions of the GM in different groups

The composition of GM at the phylum level in each sample is shown in detail (Fig. 3A). The main phyla in GM were *Firmicutes* and *Bacteroidetes*. The percentages of *Firmicutes* among all gut bacteria were 56.37 % ± 10.56 %, 54.79 % ± 11.79 %, and 51.73 % ± 9.35 % in the CI, CG, and DA groups, respectively. In contrast, the percentages of *Bacteroidetes* among all gut bacteria were 35.24 % ± 10.41 %, 39.97 % ± 9.25 %, and 35.70 % ± 12.61 % in the CI, CG, and DA groups, respectively. The F/B ratios were 1.29, 1.53, and 1.60 in the CG, DA, and CI groups, respectively (P > 0.05) (Fig. 3B). Next, to analyze the differences in microbiota composition between the CI and CG groups, as well as between the CI and DA groups, the absolute counts of taxa were tested using the DESeq2 negative binomial algorithm. The results showed that the absolute counts of the *Prevotellaceae*_NK3B31_group from the phylum *Bacteroidetes* were significantly higher in the CG than in the CI group (P = 0.004) (Fig. 3C). The absolute counts of *Pseudomonas* from the phylum *Proteobacteria* were significantly higher in the DA group than in the CI group (P = 0.000) (Fig. 3D). Finally, LEfSe was employed to identify specific genera that were differentially distributed between the CI and CG groups, as well as between the CG and DA groups. The *Muribaculaceae* lineage, both at the family and genus levels, was more enriched in the CG group than in CI group (P < 0.05, logarithm of LDA>4, Fig. 3E). The LEfSe plot comparing the DA and CI groups is shown in Fig. 3F. *Oscillospirales* at the order level were enriched in the DA group, whereas the lineage *Prevotellaceae* (family level) > *Prevotella* (genus level) > *Prevotellaceae* bacterium (species level) was enriched in the CI group (P < 0.05, logarithm of LDA>4).

3.4. Correlation of hypertension-related factors with the GM

The Spearman correlation analysis results between the vital parameters of SD rats (including HR, SBP, DBP and BW) and the levels of the main bacteria at the genus level are shown as a heatmap in Fig. 4. Based on the heatmap, we found that HR was positively correlated with *Treponema* (P = 0.038, r = 0.271) and *Lachnospiraceae_NK4A136_group* abundance (P = 0.048, r = 0.259) and negatively correlated with *Clostridia_UCG-014* abundance (P = 0.048, r = −0.259). BW positively correlated with *Lachnospiraceae_NK4A136_group* abundance (P = 0.040, r = 0.269) and negatively correlated with *Prevotellaceae_Ga6A1_group* abundance (P = 0.029, r = −0.285).

4. Discussion

In this controlled experiment with three groups of SD rats, we confirmed that continuous light exposure elevated the BP, BW, and HR of SD rats and induced changes in GM composition. Moreover, we found that inhibition of sympathetic excitation partially reversed these changes. These results indicate that CRD may harm the development of CVD by activating SN and changing GM.

Hypertention, obesity, tachycardia, metabolic disturbance and chronic inflammatory status are the risk factors for CVD. Since the 1980s, the global prevalence of obesity has shown an upward trend. Obesity directly contributes to dyslipidemia, type 2 diabetes,

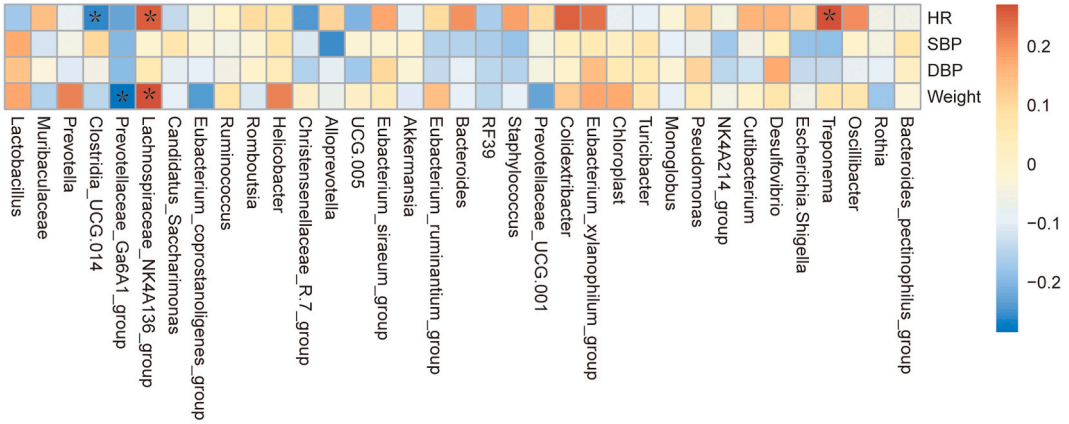

Fig. 4. Spearman correlation analysis of genera and vital parameters of Sprague Dawley (SD) rats. The colors of squares represent the r values of Spearman's correlation coefficient. *indicates significance at P < 0.05.

hypertension, and sleep disorders. It is also an independent risk factor for CVD development and increases CVD-related mortality [28]. Another important risk factor for CVD is hypertension; subtle target organ damage, such as left ventricular hypertrophy, microalbuminuria and cognitive dysfunction may occur early during the development of hypertension, whereas serious complications, such as stroke, heart attack, renal failure, and dementia, may occur if hypertension remains uncontrolled for a long time [29]. HR is another factor that is often overlooked. Paul et al. analyzed the baseline and final HR in hypertensive patients using 80 bpm as a threshold to classify patients as having a consistently high (high-high) or low (low-low) heart rate. High-high patients had a 78 % higher risk of all-cause and cardiovascular mortality than low-low patients [30]. Lin et al. found that total cholesterol level, blood glucose and serum IL-6 of rats in the continuous light group were significantly higher than those in the control group, indicating the adverse effects of continuous light on metabolism and chronic inflammation [31]. In our study, there was a statistical difference in BW between the rats in the CI group and the CG group 11 weeks after the start of the experiment. Although there was no statistical difference in BW between the rats in the continuous light group and the control group in Yue's study, the rats in the continuous light group ate less food, and they believe that continuous light may reduce the energy metabolic rate [32]. In Fonken's study, continuous light exposure significantly increased BW in mice compared to controls, which is consistent with our results [33]. Our results showed that providing continuous light to SD rats with sympathetic depressants reversed the light-induced changes in BP, BW and HR, suggesting that SN excitation might contribute to CRD-related CVD by elevating BP, BW, and HR.

A previous study demonstrated that continuous light exposure led to an increase in the relative abundance of *Firmicutes* and a decrease in the relative abundance of *Bacteroidetes* in the rat GM [34], which is consistent with our experimental results. However, our study also found that the administration of a central sympathetic depressant drug partially reversed these changes, resulting in a decrease in the relative abundance of *Firmicutes*. In addition, a study by Chu et al. showed that after four weeks of continuous light exposure in SD rats, there was no significant difference between the light-dark cycle group and the continuous light exposure group when microbial community richness (α diversity) was measured using the Chaol index [35]. The results of our experiment were similar; however, the Shannon index of the GM was higher in the DA group than in the CI group (Fig. 2C). This suggests that the inhibition of sympathetic excitation increases GM diversity in rats.

Butyric acid has been shown to protect against CVD in several studies. It ameliorated high-fat diet-induced atherosclerosis in ApoE−/− mice [36] and significantly reduced high-fat diet-induced overweight in mice [37]. Onyszkiewicz et al. demonstrated a significant hypotensive effect of an intracolonic injection of butyric acid in rats [38]. Lin et al. found that continuous light exposure resulted in reduced butyric acid levels in the colon contents of high-fat diet rats [31]. Moreover, the abundance of *Oscillospirales*, one of the most important butyric acid-producing bacteria, decreased in atherosclerotic CVD volunteers who were given one week of diet control, forced exercise, and health promotion [39]. The LEfSe results of our study showed that *Oscillospirales* were the specific bacteria altered after CH gavage. These studies suggested SN might play a role in the mechanism of Butyric acid protect against CVD.

Our experiment results also showed that the relative abundances of *Muribaculaceae*, which were among the top ten genera, were 21.73 %, 26.12 % and 25.64 % in the CI, CG and DA groups, respectively. The abundance of *Muribaculaceae* in the CI group was significantly lower than that in the CG group, and this change was partially reversed by oral administration of CH. Moreover, the LEfSe results showed that the *Muribaculaceae* family was a specific bacterial community that differed between the CG and CI groups. The *Muribaculaceae* family plays a role in propionate production [40] and is one of the main bacterial taxa in the GM of laboratory mice [40–42]. The relative abundance of *Muribaculaceae* was found to be 60 % in fecal samples from blind moles [43], and in Sibai's study of the relationship between longevity and microflora in the blind mole rat, *Muribaculaceae* was found to be a component of the core microbiome in the blind mole rat, which may explain the health and longevity of this animal. Another study showed that acarbose prolonged the lifespan of mice, with increased *Muribaculaceae* abundance in acarbose-treated mice [44]. Although our experiment did not assess and compare the lifespans of the three groups of rats, we speculate that sustained light-accelerated aging might occur in part through redistribution of the GM, and the inhibition of central sympathetic excitation might have a beneficial effect on light-accelerated aging by rebuilding the GM.

Moreover, we found that the *Prevotella* genus was the main feature discriminating bacterial communities in the CI group. Yue et al. also found that *Prevotella* was enriched in the group of rats with continuous light exposure and positively correlated with serum testosterone and IL-6 levels [45]. Li et al. showed that *Prevotella* is a common pre-hypertensive and hypertensive microbial signature that may play an important role in hypertension by triggering an inflammatory response [46]. In a study on obesity and GM changes in Korean adolescents, Hu et al. found a significant positive correlation between *Prevotella* abundance and BMI, and a positive correlation between *Prevotella* abundance and triglyceride (a risk factor for CVD) levels [46]. Increased level of *Prevotella* is associated with an inflammatory response in polycystic ovary syndrome rats and leads to hyperandrogenism [47]. At the same time, hyperandrogenemia predicts the severity of cardiometabolic profile and CVD risk [48]. These studies indicate that *Prevotella* is closely related to risk factors of CVD, such as inflammation, obesity, BP, metabolism, etc. So, we assume that *Prevotella* deficiency might reverse CVD caused by light pollution by acting on control its risk factors.

Clostridia are short-chain fatty acid (SCFA)-producing bacteria. In the context of the gut microbiota host lipid metabolism axis [49, 50], SCFAs play important roles in human health. However, *Clostridia* UCG-014 abundance was positively correlated with fasting blood glucose, total cholesterol, and BW levels [50]; Zhao et al. concluded that a decrease in *Clostridia UCG-014* abundance facilitated the return of fasting blood glucose levels to normal levels. However, the negative association between *Clostridia UCG-014* abundance and HR in this study and the fact that *Clostridia* is an SCFA-producing bacterium suggest that the role of *Clostridia UCG-014* is multifaceted and that the health benefits of simply increasing or decreasing *Clostridia UCG-014* abundance are not certain. In experiments conducted by Wang et al. among respiratory syncytial virus-infected asthmatic mice, the abundance of the *Lachnospiraceae_NK4A136_group* was significantly associated with IgE and IL-33 levels [51], and inflammatory levels were closely associated with the development of CVD. Given the correlation between high resting HR and CVD morbidity and mortality [52,53] and the fact that BMI is an independent risk

factor for CVD, we hypothesized that decreases in the levels of *Lachnospiraceae_NK4A136_group* might be beneficial for treating CVD.

5. Limitations

There were some limitations to our study: 1. The effects of exposure to extra light on organisms are complex. It not only excites the SN and alters the composition of the GM but also affects the immune, endocrine and nervous systems [54–56]. 2. Owing to experimental funding constraints, we were unable to perform continuous 16S rDNA sequencing of the rat GM, monitor GM dynamics or evaluate the relationships between these changes and SN excitation. Therefore, additional experiments are necessary to fully elucidate the contribution of the CRD to CVD.

6. Conclusions

In the present study, we found that continuous light exposure resulted in disturbances in vital signs and changes in GM composition and diversity in rats. The increase in BP, HR, and BW induced by continuous light can be reversed by the administration of sympathetic inhibitors; however, the effect of light on GM cannot be entirely eliminated. Further studies are needed to explore the mechanisms underlying the effect of continuous light on GM.

Data availability statement

Data for this paper, including vital signs and gut microbiota data are available at https://data.mendeley.com/datasets/64z84btr5c/2.

CRediT authorship contribution statement

Yi Zhao: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Xu-ming Ma:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - original draft, Writing - review & editing. **Ming Ren:** Data curation, Formal analysis. **Huiqin Liu:** Formal analysis, Methodology. **Hao-liang Duan:** Investigation, Software. **Xing-li Liu:** Conceptualization, Visualization. **Zhong-shan Gao:** Validation, Writing - original draft. **Yu-lan Ma:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22742.

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