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Investigation on the influence of isolated environment on human psychological and physiological health

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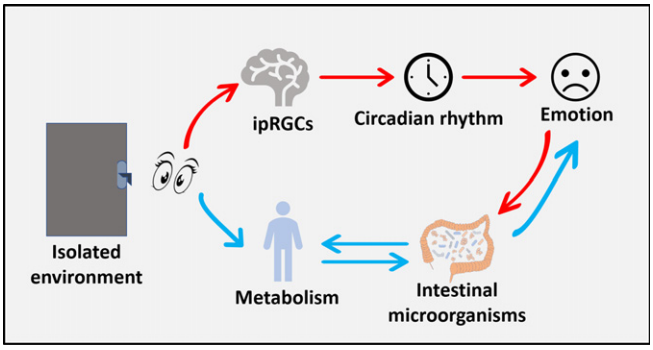
HIGHLIGHTS

- Crewmembers have no psychological pain and physiological obstacles in LP1.
- Lacking natural light and perception may increase the incidence of ANX and DEP.
- Lacking natural light and perception influence tryptophan metabolism pathway.
- *Prevotella* may play a critical role in health regulation in isolated environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Crewmembers are working and living in isolated environment lacking natural light and perception. Although their health problems have been documented, the mechanism has not been thoroughly investigated. The aim of the present study is to investigate the psychological and physiological influences of isolated environment on crewmember's health. On account of complexity of the isolated environment, it is necessary to have a manually controllable system to simulate research platform-Bioregenerative Life Support System (BLSS). Symptom checklist 90 (SCL-90) was used to complete emotional status test. Urine samples were collected for metabonomics and hormone secretion analysis. Fecal samples were collected for intestinal microorganisms analysis. Crewmembers ($n = 4$) followed strict daily schedule during the experimental period. Five emotional factors were significantly ($P < 0.05$) increased, differential metabolites were enriched in tryptophan metabolism pathway, the relative abundance of *Prevotella* decreased significantly ($P < 0.0001$) when crewmembers in isolated environment without natural light. Hormone (melatonin, cortisol) secretion rhythm also changed. Significant positive correlation ($r = 0.805$, $P < 0.05$) between cortisol secretion and anxiety was observed. In conclusion, natural light simulation in an isolated environment may have a positive effect on the physiological and psychological health of the crewmember.

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1. Introduction

As scientific and technological activities increase, such as manned space flight, submarine military mission, flight and landing tests of reusable suborbital systems, space tourism and space science activities, the number of participants will also increase significantly. Human health status in the isolated environment becomes more and more important in continuous operation time and operation performance (Barshi and Healy, 2002; Keyak et al., 2009; Williams, 2015; Wang, 2011).

Light is an important regulator of physiology and behavior, affecting various functions such as daily activity rhythms, sleep and melatonin synthesis (Herzog, 2007). A new type of nerve cell-intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) that express melanopsin are mainly distributed in the retina of the eye (Berson, 2003). Rod cells and cone cells in the retina convert light energy into electrical signals, and transmit both image and non-image forming information through ipRGCs. There is a neural projection between ipRGCs and suprachiasmatic nucleus (SCN) that send to the brain (Berson, 2003; Hankins et al., 2008; Hattar et al., 2002). ipRGCs affect the pineal gland located in the hypothalamus directly, subsequently influence on melatonin expression that related to biological rhythm and has made wide effects on aging, sleep, cancer and so on (Chen et al., 2011; Fernandez et al., 2018; Jin et al., 2018). Normal light environment is good for reaction time, memory ability and visual accommodation (Fujioka et al., 2011; Gibson et al., 2010; Lamprecht, 2019), performing an increasingly vital role in human health.

However, one of the main challenges is that the isolated environment is lack of natural light and perception. Several previous studies have reported that abnormal light environment is related to colorectal cancer, prostate cancer, seasonal affective disorder, circadian rhythm and so on (Boivin and Boudreau, 2014; Figueiro et al., 2014; Legates et al., 2014; Schernhammer et al., 2003; Sher, 2000). Therefore, crewmembers' metabolism and hormone secretion was detected firstly. Meanwhile, more and more studies have reported that intestinal flora is related to anxiety, depression, stress and cognitive changes over the past few years, but the causal relationship between them are still being explored (Foster and Neufeld, 2013; Maehata et al., 2019; Wang and Kasper, 2014). With the rapid development of metabonomics and high-throughput sequencing technology, human metabolic and intestinal health problems can be explored thoroughly (Nicholson and Lindon, 2008; Oulas et al., 2015).

The influences of lacking natural light on human body have been studied. For example, in high latitudes that lacking natural light can easily lead to "seasonal emotional disorders" with special symptoms such as depression, irritability, increased sugar metabolism and so on. (Levitan, 2007; Sher, 2000; Sit et al., 2011). Studies suggested that "seasonal emotional disorders" are mainly due to lacking sunlight exposure leading to the abnormal melatonin secretion rhythm, which result in the biological rhythm disorder (Arendt, 2012; Lewy et al., 2006; Melrose, 2015; Tam et al., 1995).

In addition, Wang et al. summarized the clinical characteristics and possible mechanisms of gastrointestinal problems in modern wars (Wang et al., 2015). Wu et al. reported the problem of astronauts on orbit sleep and its countermeasures (Wu et al., 2018). Although studies about the effects of light on human health have been done, a common problem in these studies is that there are too many factors may be affecting the study. Secondly, these studies are still in their preliminary, the mechanism has not been explored thoroughly. This study needs a highly isolated system to simulate the environment of manned space flight, submarines and so on.

It is worth noting that the bioregenerative life support system (BLSS), an artificial ecosystem composed of biological and non-biological factors, is an isolated system different from other life support systems (Dempster et al., 2004; Fu et al., 2016; Gitelson et al., 1989). Lunar Palace 1 (LP1), one of the latest developments of BLSS, could satisfy the requirements of this study purpose with a closure coefficient of 97% (Dong et al., 2015; Fu et al., 2016; Hao et al., 2018). Considering the possible interfering factors, such as rigorous isolated environment, environmental microorganisms, diet, daily schedule and so on. The following characteristics of LP1 can avoid these potential interfering factors: (1) LP1 has no quality exchange with outside, which can maintain the relative stability of environmental microorganisms to the maximum; (2) crewmembers have a prescribed diet and the same food source; (3) crewmembers lives a law of life and strictly followed the prescribed daily schedule in LP1.

This study aimed to explore how lacking natural light and perception influence crewmembers' emotional, metabolic and intestinal health in isolated environment. Studies have reported that disturbed intestinal microorganisms reach a new balance needing about 21 days, and the metabolism is about 21 days (Bloomer et al., 2011; Bohoněk et al., 2009; Sommer et al., 2017). Considering the regulation of human ecosystem and the closed environment, we chose 3 weeks per stage. This study was conducted at three stage: isolated environment with natural light and perception (Stage1, P0, 3 weeks, 127–148 days), isolated environment without natural light and perception (Stage2, P1, 3 weeks, 149–170 days), isolated environment with abnormal natural light and perception (Stage3, P2, 3 weeks, 171-192 days).

2. Materials and methods

2.1. Experimental design

Four Chinese crewmembers completed this study. Crewmember A (female), Crewmember B (female), Crewmember C (male), Crewmember D (male) were the crewmember of "Lunar Palace 365 experiment" in the LP1 (http://www.lss-lab.bme.buaa.edu.cn/), the average age is 26. A complete physical examination was performed for four crewmembers, and indices were in normal range (performed at the 306th Hospital of PLA, Beijing, China). During "Lunar Palace 365 experiment", the crewmembers' daily schedule, working intensity and daily diet were strictly regulated. LP1 consist of two separate plant cabins ($10 \times 6 \times 3.5$ m^3) and a comprehensive cabin ($14 \times 3 \times 2.5$ m^3), which include a waste treatment room, a bathroom and a living room with four crewmember bedrooms. This study began ninety days after crewmembers entered the LP1 system. More intuitive experimental design and information as shown in Fig. 1.

2.2. Crewmembers' emotion states

Four crewmembers' emotion states were recorded using the Symptom checklist 90 (SCL-90) (SCL-90) questionnaires. SCL-90, as a descriptive measure of psychopathology, is a 90-item self-report symptom inventory (Derogatis et al., 1973). SCL-90 has been widely used as a brief indicator of emotional health (Chen et al., 2014; Rytilamanninen et al., 2016; Schmitz et al., 1999). It is intended to measure symptom intensity on nine different subscales including somatization (SOM), obsessive compulsive (O-C), interpersonal sensitivity (I-S), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR), and psychoticism (PSY) (Derogatis et al., 1973; Evenson et al., 1980). Each of the subscales comprises 6–13 items, the factor scores on each subscale are means of the scores of all items of the dimension (Derogatis et al., 1973; Evenson et al., 1980). Crewmembers were asked to self-identify whether each item was descriptive of themselves at the time, the 90 items was scored on a five-point scale ranging from "not at all" to "extremely". In this study, we obtained 72 questionnaire profiles from four crewmembers (two times a week).

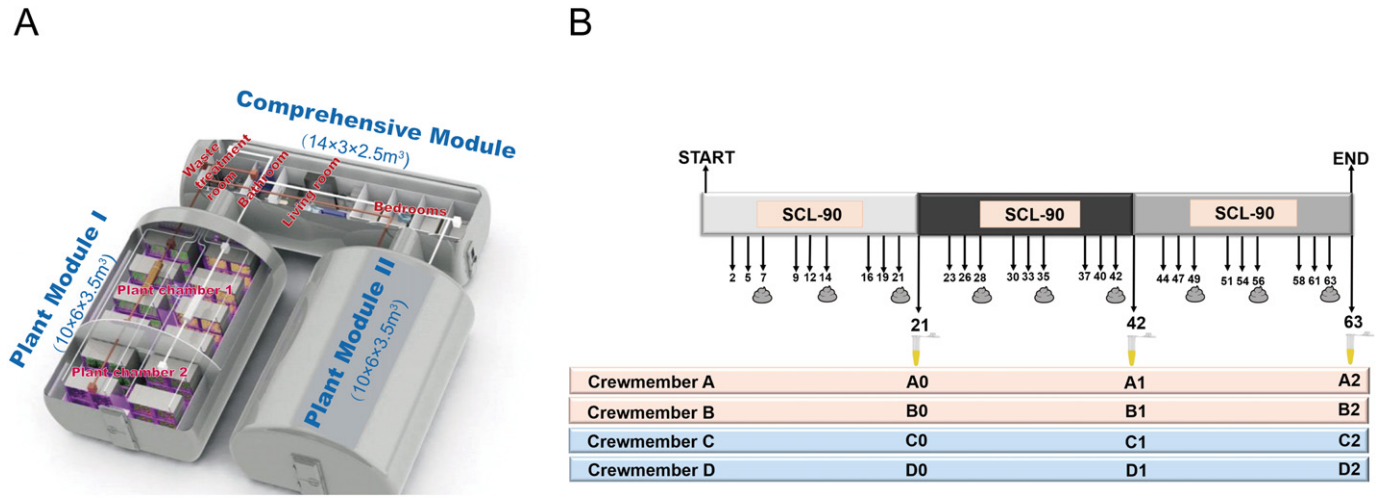


Fig. 1. Experimental design and procedures. (A) Structure of "Lunar Palace 1". Crewmembers filling in the SCL-90 and having dinner in LP1. (B) Experimental design for the 63-day timeline. Urine samples were collected on days 20–21, 41–42 and 62–63. SCL-90 was filled in twice a week; stool samples were collected on the 7th, 14th, 21st, 28th, 35th, 42nd, 49th, 56th and 63rd days. Note. A0/B0/C0/D0, P0 stage.; A1/B1/C1/D1, P1 stage; A2/B2/C2/D2, P2 stage; SCL-90, symptom checklist 90.

2.3. Metabolite profiling

2.3.1. Metabolites extraction

12 urine samples in the last 48 h of each stage were collected and homogenized. Homogenate was resuspended with prechilled 80% methanol and 0.1% formic acid by fully vertexing. Samples were incubated on ice for 5 min and then were centrifuged at 15,000 rpm, 4 °C for 5 min. Supernatant was diluted to final concentration containing 60% methanol by liquid chromatography–tandem mass spectrometric (LC-MS) grade water. Samples were transferred to a fresh Eppendorf tube with 0.22 μm filter and then were centrifuged at 15,000*g*, 4 °C for 10 min (Ohta et al., 2009; Sellick et al., 2011). Finally, the filtrate was injected into the LC-MS system analysis.

2.3.2. LC-MS analysis

Vanquish UHPLC system (Thermo Fisher) coupled with an Orbitrap Q Exactive HF-X mass spectrometer (Thermo Fisher) were using. Quality control (QC) samples were used to determine the state of the instrument before injection and balance the system, and to evaluate the stability of the system during the experiment. QC was to take an equal amount of per urine sample and mix it into a quality control samples. Samples were injected onto an Hyperil Gold column (100 × 2.1 mm, 1.9 μm) using a 16-min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive polarity mode were eluent A (0.1% FA in Water) and eluent B (Methanol). The solvent gradient was set as follows: 2%B, 1.5 min; 2–100%B, 12.0 min; 100%B, 14.0 min; 100–2%B, 14.1 min; 2%B, 16 min. Q Exactive HF-X mass spectrometer was operated in positive/negative polarity mode with spray voltage of 3.2 kV, capillary temperature of 320 °C, sheath gas flow rate of 35 arb and aux gas flow rate of 10 arb. The raw data files generated by LC-MS were processed using the Compound Discoverer 3.0 (CD 3.0, Thermo Fisher) to perform peak alignment, peak picking, and quantitation for each metabolite. The main parameters were set as follows: retention time tolerance, 0.1 min; actual mass tolerance, 5 ppm; signal intensity tolerance, 30%; signal/noise ratio, 3; and minimum intensity, 100,000. After that, peak intensities were normalized to the total spectral intensity. The normalized data was used to predict the molecular formula based on additive ions, molecular ion peaks and fragment ions. And then peaks were matched with the mzCloud and ChemSpider database to obtain the relative quantitative results.

2.4. Melatonin and cortisol measurement

Crewmembers' urine samples were obtained at three time points (7:00 am, 15:00 pm and 23:00 pm), and stored at −20 °C until analysis. Melatonin and cortisol levels measured at laboratory using the 6HMS enzyme-linked immunosorbent assay (ELISA) kit (Beijing Rigor Bioscience Development LTD, Beijing, China) and the cortisol enzyme-linked immunosorbent assay (ELISA) kit (Beijing Rigor Bioscience Development LTD, Beijing, China) according to the manufacturer's protocol. Briefly, add biotin antigen working solution, incubate 1 h, wash 5 times. Secondly, add avidin horseradish peroxidase, incubate 1 h, wash 5 times. Then, add chromogenic reagent A and B. Finally, add termination solution to test sample. We perform three tests per sample and took the mean value.

2.5. Information analysis of metagenomic

2.5.1. DNA extraction and detection

Fresh stool samples were collected from each crewmember every week (9 time points) and 36 samples were collected. At least 1 g of homogeneous fresh stool samples were collected in a self-made sample collection tube containing sample preservation solution and stored quickly at −80 °C until analysis. When all samples were collected, DNA extraction was performed (Gorzelak et al., 2015; Song et al., 2016). Firstly, stool samples were thawed on ice and DNA extracted by method of phenol/trichloromethane/isoamyl alcohol (Kumar et al., 2016; Wu et al., 2019). The extract was treated with DNase-free RNase to eliminate RNA contamination. The quality of DNA and DNA concentrations was determined by Nanodrop (Thermo-Scientific). The molecular size of DNA was determined by agarose gel electrophoresis.

2.5.2. DNA library construction and sequencing

The DNA library construction was performed following the manufacturer's instruction (Illumina). The workflow was cluster generation, template hybridization, isothermal amplification, linearization, blocking and denaturation, and hybridization of the sequencing primers (Qin et al., 2012). Paired-end metagenomics sequencing was performed on the BGI-SEQ500 platform (insert size 350 bp, read length 100 bp), each sequencing run had 8 samples and an average of 13.3 Gb (ranging between 8.23 and 21.3 Gb) data was generated for each sample.

2.5.3. Sequencing results pretreatment

Raw data was conducted to acquire the Clean Data for subsequent analysis using Readfq (V8, https://github.com/cjfields/readfq). Steps are as follows: a) remove the reads which contain low quality bases above a certain portion (length of 40 bp); b) remove the reads in which the N base has reached a certain percentage (length of 10 bp); c) remove reads which shared the overlap above a certain portion with Adapter (length of 15 bp). Clean Data need to be blast to the host database using Bowtie2.2.4 software (Bowtie2.2.4, http://bowtiebio.sourceforge.net/bowtie2/index.shtml) to filter the reads that are of host origin.

2.5.4. Metagenome assembly

The Clean Data was assembled and analyst by SOAPdenovo software (Feng et al., 2015; Luo et al., 2012). Then interrupted the assembled Scaftigs from N connection and left the Scaftigs without N (Mende et al., 2012; Nielsen et al., 2014; Qin et al., 2014). All samples' Clean Data were compared to each Scaffolds respectively by Bowtie2.2.4 software to acquire the PE reads not used (Qin et al., 2014). All the reads not used in the forward step of all samples were combined and then used the software of SOAPdenovo (V2.04)/MEGAHIT (v1.0.4-beta) for mixed. Break the mixed assembled Scaffolds from N connection and obtained the Scaftigs. Filter the fragment shorter than 500 bp in all of Scaftigs for statistical analysis.

2.5.5. Gene prediction and abundance analysis

The Scaftigs (≥ 500 bp) assembled from both single and mixed were all predicted the ORF by MetaGeneMark (V2.10) software, and filtered the length information shorter than 100 nt from the predicted result with default parameters (Nielsen et al., 2014; Qin et al., 2010; Qin et al., 2014). For ORF predicted, CD-HIT software (V4.5.8) was adopted to redundancy and obtain the unique initial gene catalogue (Fu et al., 2012; Li and Godzik, 2006).

The Clean Data of each sample was mapped to initial gene catalogue using Bowtie2.2.4 and get the number of reads to which genes mapped in each sample (Li et al., 2014; Qin et al., 2014). Filtered the gene which the number of reads ≤2 in each sample and obtained the gene catalogue (Unigenes) eventually used for subsequently analysis (Li et al., 2014; Qin et al., 2012). Based on the number of mapped reads and the length of gene, statistic the abundance information of each gene in each sample. The format is as follow, r represents the number of reads mapped to the genes and L represents gene's length (Nielsen et al., 2013; Villar et al., 2015). Then, the core-pan gene analysis was based on the abundance of each gene in each sample in gene catalogue.

$$G_k = \frac{r_k}{L_k} \cdot \frac{1}{\sum_{i=1}^{n} \frac{r_i}{L_i}}$$

2.5.6. Taxonomy prediction

DIAMOND software (V0.9.9) was used to blast the Unigenes to the sequences of Bacteria, Fungi, Archaea and Viruses which were all extracted from the NR database (Version: 2018-01-02) of NCBI (Buchfink et al., 2015).

For the finally aligned results of each sequence, as each sequence may have multiple aligned results, choose the result of which the e value ≤ the smallest e value*10 to take the LCA algorithm which was applied to system classification of MEGAN software to make sure the species annotation information of sequences, Krona analysis to exhibit the generation situation of relative abundance (Avershina et al., 2013; Buchfink et al., 2015; Huson et al., 2011).

2.6. Statistical analysis

Prior to determining the significance with parametric tests, normality was tested using D'Agostino-Pearson omnibus normality test. For normally distributed data, significance was determined using unpaired two-tailed Student's *t*-test. Mann-Whitney test was used when data failed the normality test (GraphPad Software, Inc., La Jolla, CA, USA). Histograms and line charts were presented with means ± standard deviation (SD). $P < 0.05$ was considered to indicate a statistically significant difference. Differences were noted as significant $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Statistical analysis was carried out using Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Correlations were analyzed with Pearson's correlation coefficient (PCC). The software of R language (Version 3.5.0) was used in Fig. 3A (Team, 2014). Pearson correlation coefficient was used to assess the urine data quality. Before Pearson correlation test, the quantitative value of the data in Fig. 3A was processed by log10 (Peak. Area + 1). Data in Fig. 3B was processed by log10 (Peak. Area), the expression and density distribution of each metabolite can be seen. PCA was performed using the ade4 function in the R package for calculation. Volcanic map was the base logarithm of the difference multiple of each metabolite with 2 as the base, and the absolute logarithm of *P*-value with 10 as the base. PCA and volcanic maps were conducted to assess the variability in general and to analyze the general distribution tendency among samples. Hypergeometric test was used in Fig. 3G-H to identify which pathway was significantly enriched in the differential metabolites. KEGG enrichment bubble chart was the ratio of the number of differential metabolites in the corresponding pathway to the number of total metabolites identified (see Fig. 3G-H).

2.7. Ethics statement

Experiments were performed with the consent of crewmember and with approval by the Science and Ethics Committee of School of Beihang University (Approval ID: BM20180003).

3. Results and discussion

3.1. Factor scores of anxiety and depression increased significantly in an isolated environment without natural light and perception

During “Lunar Palace 365 experiment”, crewmembers' diet was based on National Aeronautics and Space Administration (NASA) astronauts' dietary standards. Most of crewmembers' health indicators were within normal range and there were no health problems. In present study, SCL-90 was used to evaluate crewmembers' emotion and assess the influence of isolated environment on crewmembers. The emotion of four crewmembers swinged within a certain range (Depression, 1–1.92; Anxiety, 1–1.90), but all the factor scores did not exceed 2.0, which indicated crewmembers' emotional state was keeping good (see Fig. 2A-D).

This was consistent with previous data that all crewmembers' emotion factor scores were within normal range with no obvious psychological distress or turbulence (Hao et al., 2018). Comparing the score of each subscale of four crewmembers, significant differences was observed in subscale of interpersonal sensitivity ($P = 0.0238$), depression ($P = 0.0027$), anxiety ($P = 0.0249$), hostility ($P = 0.0028$) and phobic anxiety ($P = 0.0373$) between P0 and P1 stage (see Fig. 2E). No significant difference ($P > 0.05$) was observed in the number of positive items among different stage (see Fig. 2F).

In conclusion, emotional swings were occurred when crewmembers in an isolated environment without natural light and perception. More importantly, we found that the scores of I-S, DEP, ANX, HOS, PHOB at P1 stage were significantly higher than those at P0 stage ($P < 0.05$). These results indicated that lacking natural light and perception in an isolated environment may increase the incidence of ANX and DEP.

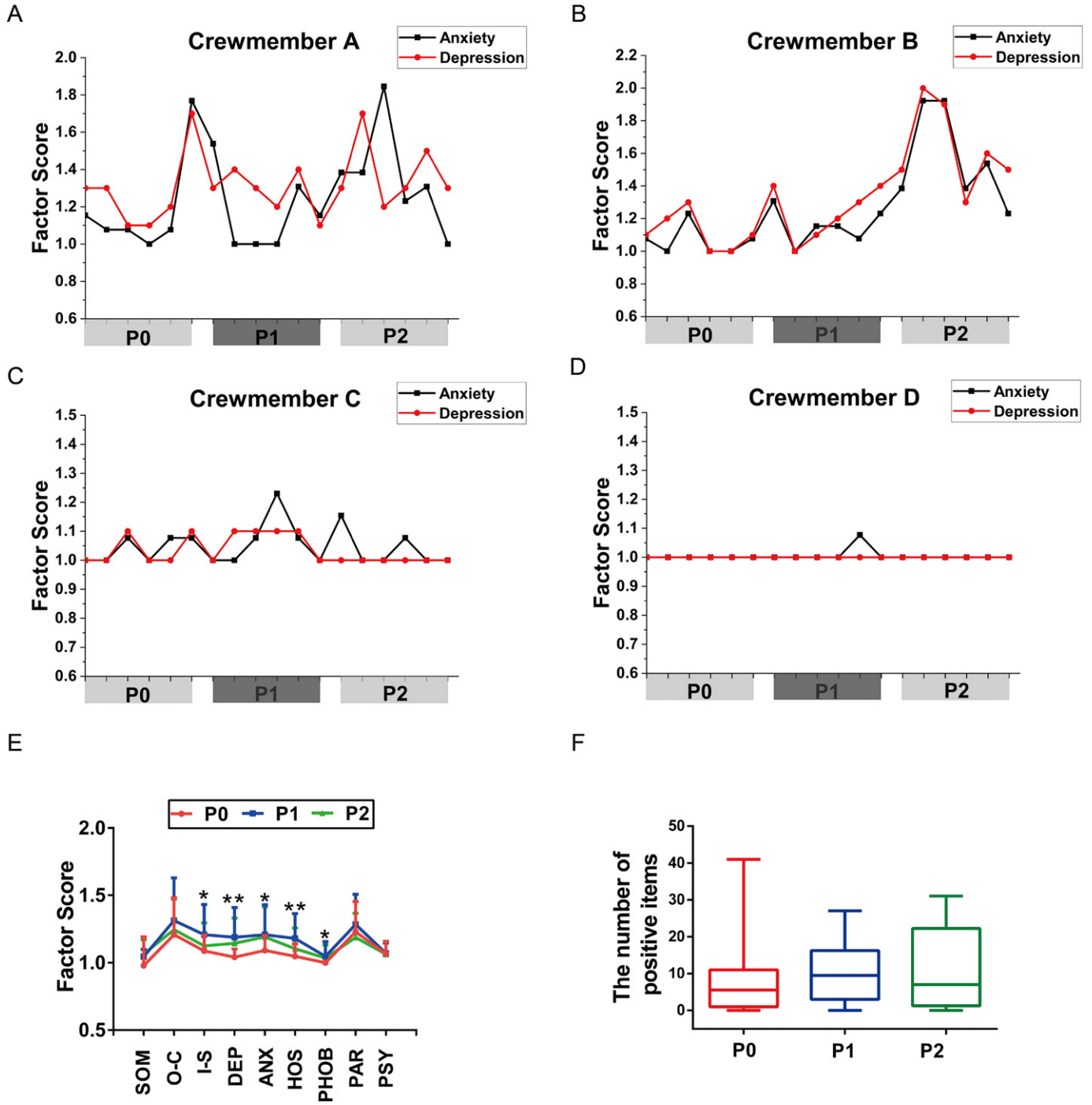
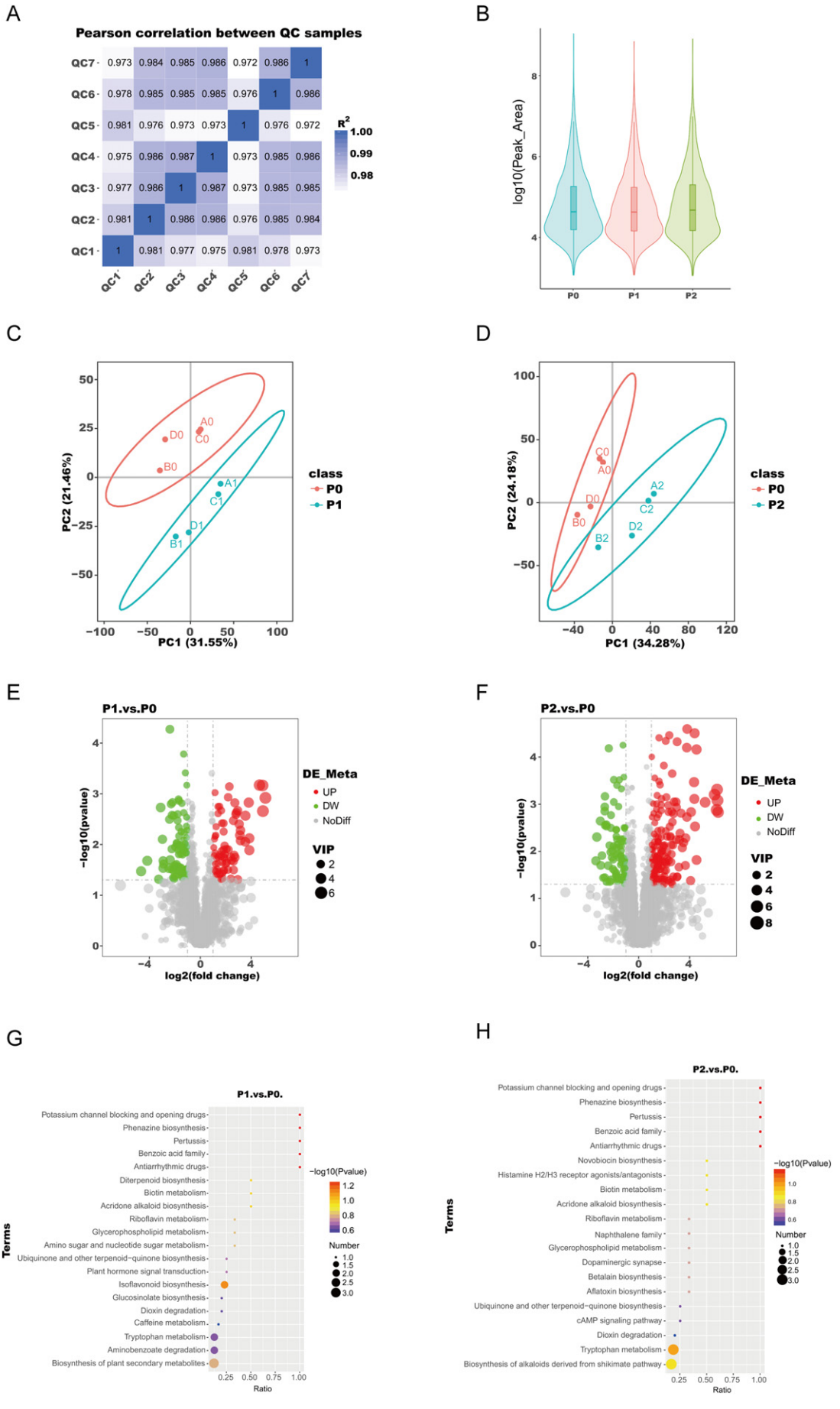


Fig. 2. Four crewmembers' emotional status. Crewmembers' (A-D) factor score of SCL-90 subscale at three stages (P0, P1, P2); nine subscales scores of SCL-90 at three stages (E); Difference in the number of positive items (F). Unpaired *t*-test was conducted to compare group differences. Note. $^{*}P < 0.05$, $^{**}P < 0.01$; Data in panels A-D is expressed as Mean value, data in panels E-F is expressed as Mean ± SD; SOM, somatization; O-C, obsessive-compulsive; I-S, interpersonal sensitivity; DEP, depression; ANX, anxiety; HOS, hostility; PHOB, phobic anxiety; PAR, paranoid ideation; PSY, psychoticism; P0, isolated environment with normal natural light and perception; P1, isolated environment without natural light and perception; P2, isolated environment with abnormal natural light and perception.

3.2. Significant influence on metabolism was observed in isolated environment without natural light and perception

In order to investigate thoroughly above findings, we analyzed crewmembers' metabolome and metabolites expression. QC (n = 7) analysis showed the correlation coefficients of QC samples were almost one ($R^2 > 0.97$), which indicating metabolomic data had the least error (see Fig. 3A). As Fig. 3B shown, the expression level of general metabolites in three different isolated light environments had no significant difference ($P > 0.05$). Visualization of the metabolites composition by PCA, samples were obviously separated between P0 stage and P1 stage, which indicated the metabolites profile differed significantly between P0 stage and P1 stage (see Fig. 3C). The 95% confidence interval had some overlapping regions between P0 stage and P2 stage (see Fig. 3D). Compared with P0 stage, crewmembers at both P1 and P2 stage all have up and down expression metabolites (see Fig. 3E-F). It should be noted that the differential metabolites were enriched in tryptophan metabolism pathway after crewmember entered the P1 stage (see Fig. 3G-H).


A
Pearson correlation between QC samples
QC7 0.973 0.984 0.985 0.986 0.972 0.986 1
QC6 0.978 0.985 0.985 0.985 0.976 1 0.986
QC5 0.981 0.976 0.973 0.973 1 0.976 0.972
QC4 0.975 0.986 0.987 1 0.973 0.985 0.986
QC3 0.977 0.986 1 0.987 0.973 0.985 0.985
QC2 0.981 1 0.986 0.986 0.976 0.985 0.984
QC1 1 0.981 0.977 0.975 0.981 0.978 0.973
QC1 QC2 QC3 QC4 QC5 QC6 QC7
R2
1.00
0.99
0.98
B
log10(Peak_Area)
P0 P1 P2
C
PC2 (21.46%)
PC1 (31.55%)
A0 D0 C0 B0 A1 C1 D1 B1
class
P0
P1
D
PC2 (24.18%)
PC1 (34.28%)
C0 A0 D0 B0 A2 C2 D2 B2
class
P0
P2
E
P1.vs.P0
−log10(pvalue)
log2(fold change)
DE_Meta
UP
DW
NoDiff
VIP
2
4
6
F
P2.vs.P0
−log10(pvalue)
log2(fold change)
DE_Meta
UP
DW
NoDiff
VIP
2
4
6
8
G
P1.vs.P0.
Potassium channel blocking and opening drugs
Phenazine biosynthesis
Pertussis
Benzoic acid family
Antiarrhythmic drugs
Diterpenoid biosynthesis
Biotin metabolism
Acridone alkaloid biosynthesis
Riboflavin metabolism
Glycerophospholipid metabolism
Amino sugar and nucleotide sugar metabolism
Ubiquinone and other terpenoid−quinone biosynthesis
Plant hormone signal transduction
Isoflavonoid biosynthesis
Glucosinolate biosynthesis
Dioxin degradation
Caffeine metabolism
Tryptophan metabolism
Aminobenzoate degradation
Biosynthesis of plant secondary metabolites
Terms
Ratio
−log10(Pvalue)
Number
H
P2.vs.P0.
Potassium channel blocking and opening drugs
Phenazine biosynthesis
Pertussis
Benzoic acid family
Antiarrhythmic drugs
Novobiocin biosynthesis
Histamine H2/H3 receptor agonists/antagonists
Biotin metabolism
Acridone alkaloid biosynthesis
Riboflavin metabolism
Naphthalene family
Glycerophospholipid metabolism
Dopaminergic synapse
Betalain biosynthesis
Aflatoxin biosynthesis
Ubiquinone and other terpenoid−quinone biosynthesis
cAMP signaling pathway
Dioxin degradation
Tryptophan metabolism
Biosynthesis of alkaloids derived from shikimate pathway
Terms
Ratio
−log10(Pvalue)
Number

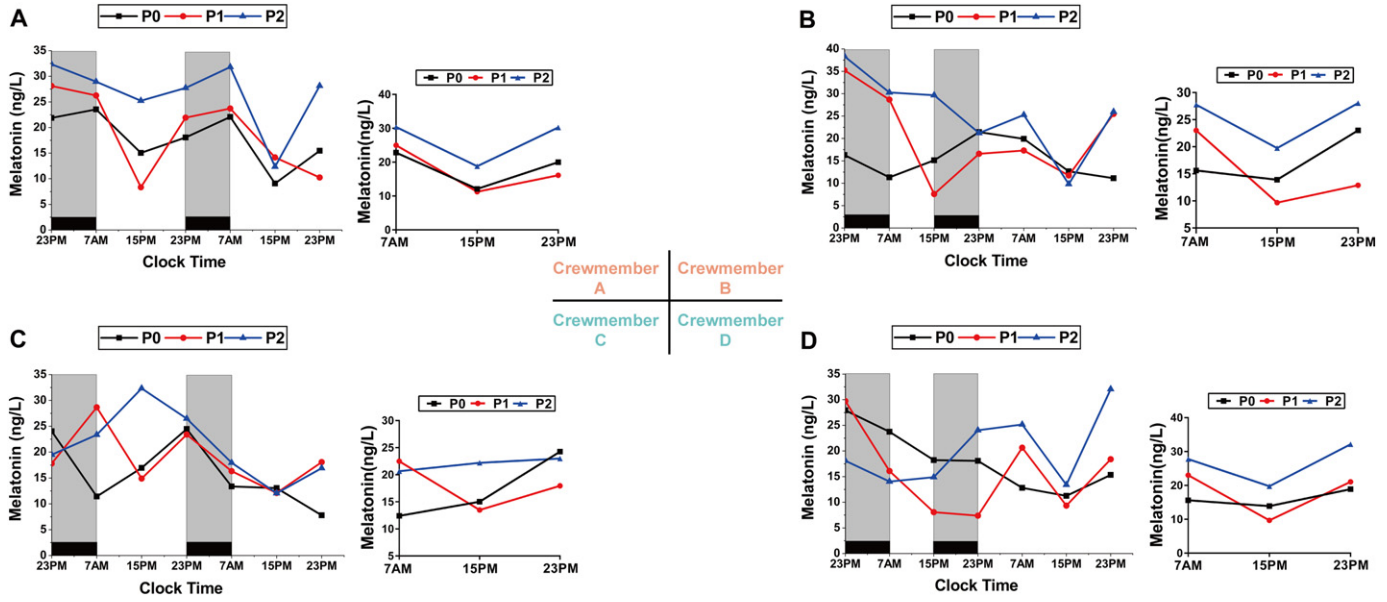


Fig. 4. Changes in melatonin secretion at different times of the day. Melatonin secretion level of crewmembers (A-D) in the last 48 h of each stage were analyzed. The gray periods were night time, while the unmarked periods were day time. Samples measured were in dim light. Data is expressed as Mean.

Tryptophan, which can cross the blood-brain barrier, is converted to 5-hydroxytryptophan (5-HTP) by hydroxylase and then be converted to 5-hydroxytryptamine (5-HT) by decarboxylase in vivo (Barnes and Sharp, 1999; Jenkins et al., 2016; Kokturk and Kanbay, 2015). Previous studies reported that 5-HT regulates human emotion and enhances memory (Jenkins et al., 2016). In addition, 5-HT also produce melatonin via *N*-acetyl-5-hydroxytryptamine (Barnes and Sharp, 1999; Kokturk and Kanbay, 2015). Endogenous melatonin is a hormone secreted by pineal gland and involved in regulating the circadian rhythm and the physiological function of sleep (Armstrong, 1989; Claustrat and Leston, 2015; Pandiperumal et al., 2006). Above results suggested that emotional swing may be related to tryptophan metabolism pathway. It can be concluded that lacking natural light and perception in isolated environment might have an impact on human metabolism.

3.3. Effect of isolated environment without natural light and perception on the hormonal secretion rhythm

We examined the dynamic changes of melatonin and cortisol secretion in different isolated light environments. Differences in the melatonin and cortisol secretion among four crewmembers can be seen in Figs. 4A-D and 5A-D. In different isolated light environments, four crewmembers' melatonin secretion level showed a circadian variation that low levels (13.93 ± 3.41 ng/L) during the day and high levels (21.53 ± 3.40 ng/L) at midnight (see Fig. 4A-D). Zamanian et al. reported that with the increase of light intensity, the level of melatonin in plasma decreased (Zamanian et al., 2013). Melatonin secretion level began to rise and gradually accumulate after light weakened at night, then declined in the morning, and reached the lowest level in the afternoon, and changed with the variation of light intensity (Veen et al., 2009). However, by observing the dynamic variation of melatonin secretion, we found that there was a delayed peak phase in general when crewmembers were in the absence of natural light and perception environment (see Fig. 6A), indicating natural light perception in isolated environment may be beneficial to melatonin secretion rhythm.

In terms of cortisol levels, which general follows the rhythm with high levels (38.61 ± 13.72 ng/mL) in the morning and the lowest levels (14.41 ± 6.14 ng/mL) at midnight (see Fig. 6B). The peak value of cortisol secretion in the P1 stage was higher than P0 stage (crewmember A, 43.06 ng/mL vs 27.42 ng/mL; crewmember B, 96.85 ng/mL vs 55.43 ng/mL; crewmember C, 51.45 ng/mL vs 44.88 ng/mL; crewmember D, 60.07 ng/mL vs 43.16 ng/mL) (see Fig. 5A-D). The cortisol secretion peak value of crewmember B at P1 stage was almost twice as high as that at P0 stage (see Fig. 5B). In addition, we also evaluated whether there were a sex differences between females and males in the secretory rhythm and secretory level of melatonin and cortisol. As shown in Figs. S1A-C and S2A-C, there was no significant sex difference in melatonin and cortisol secretion levels. However, the cortisol secretion rhythm in female was affected by the presence of natural light perception in an isolated environment.

Previous studies have reported that depressive patients had high level of cortisol (Beckfriis et al., 1985; Fischer et al., 2017; Heranevives et al., 2018; Owens et al., 2014; Zvěřova et al., 2013). To verify whether cortisol level was related to healthy human emotional status, Pearson correlation analysis was conducted between scores of each factor and cortisol secretion level at three time points. Significant and positive correlation between cortisol level and anxiety ($r = 0.805$; $P < 0.05$) at P0 stage was observed (see Fig. 6C).

Jung et al. found that the plasma cortisol level was significantly reduced by strong light irradiation, while the cortisol level was almost not affected by weak light irradiation (Jung et al., 2010). Gunn et al. reported that under constant routine conditions, the circadian secretion of melatonin and cortisol in urine was not significantly different between females and males (Gunn et al., 2016). However, plasma melatonin and cortisol levels were significantly higher in female than in male, but there was no difference in cortisol levels between females and males (Gunn et al., 2016).

Fig. 3. Metabolome analysis of four crewmembers. (A) QC sample (n = 7) quality control. R^2: Pearson correlation coefficient, QCx: quality control. (B) The quantitative values of metabolites; PCA (C-D) plot based on Bray-Curtis distances and the confidence ellipse is 95%; Volcanic maps (E-F), the abscissa represents the differential multiple of differential metabolites (log2 value), the vertical axis represents the *P*-value (−log10 value); Bubble map of the enriched KEGG pathway (G-H) (showing the results of top20). The abscissa is the ratio of the number of differential metabolites to the total number of identified metabolites in the corresponding pathway. The color of dots represents the p-value value of the hypergeometric test. The size of the dots represents the number of differential metabolites in the corresponding pathway.

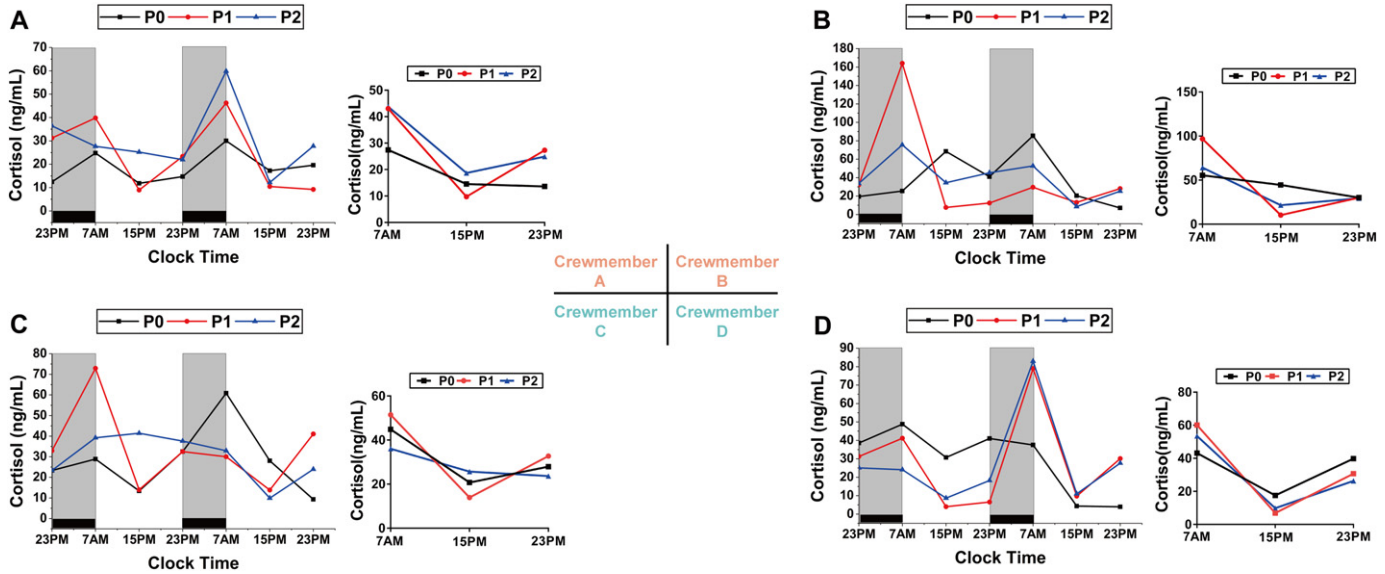


Fig. 5. Changes in cortisol secretion at different times of the day. Cortisol secretion level of crewmembers (A-D) in the last 48 h of each stage were analyzed. The gray periods were night time, while the unmarked periods were day time. Samples measured were in dim light. Data is expressed as Mean.

This is in concert with our results, daylight inhibited cortisol secretion and cortisol level began to rise at night when there was almost no light (Jung et al., 2010). From above results, it can be concluded that the isolated environment without natural light and perception may influence the rhythm of hormone secretion, which may then affect human emotional status. So, we believe that providing normal natural light and perception in isolated environment has a beneficial on the hormone rhythmic secretion, and also on reducing cortisol secretion contributing to crewmembers' stability of positive emotion.

3.4. Isolated environment without natural light and perception decreased the relative abundance of Prevotella

Voorhies et al. found that during the mission of the international space station (ISS), the composition of astronauts' microbial community changed (Voorhies et al., 2019). In order to understand the intestinal microorganisms in different isolated light environment, we collected crewmembers' feces for high-throughput sequencing and investigated the microbial community composition. A total of 306,157.29Mbp raw data (average 8504.37Mbp) was obtained from 36 feces samples. After removing the low-quality data and host data, 303,449.94Mbp nohost data was determined to the following analysis with an average 8429.17Mbp per sample, and the GC contents were 47.39%.

Gene rarefaction curves showed that feasible data quality and sequencing depth (see Fig. 7). To better understand the differences in composition of intestinal microbiota between different isolated light environment, the taxonomic annotation was analyzed and as shown in Fig. 8A-B. As shown in Fig. 8A, the phylum level analysis demonstrated that with or without natural light had little effect ($P > 0.05$) on the relative abundance of Bacteroidetes and Firmicutes in an isolated environment. In the generic level, isolated environment without natural light and perception significantly ($P < 0.001$) decreased the relative abundance of *Prevotella* (see Fig. 8B).

Prevotella is an important genus of *Bacteroides* (Zhou and Li, 2015). Studies have showed that dietary fiber intake improving glycometabolism is associated with increased abundance of *Prevotella* (De Filippis et al., 2016; Kovatchevadatchary et al., 2015; Makki et al., 2018). Zhao et al. reported that high-fiber diets promote the growth of intestinal bacteria producing short-chain fatty acids (Zhao et al., 2018). Short-chain fatty acids create a mild acidic intestinal environment, which assist intestinal reducing the number of harmful bacteria. The dietary structure of crewmembers in “Lunar Palace 365 experiment” was high dietary fiber. In addition, it is worth noting that Petersen et al. have demonstrated that the transcriptional activity of *Prevotella* is negatively correlated with the tryptophan metabolism pathway. In other words, the higher the relative abundance of *Prevotella*, the lower the tryptophan metabolism activity (Petersen et al., 2017). The metabolomic analysis of present study showed that differential metabolites were enriched in tryptophan metabolism pathway when crewmembers in isolated environment without natural light and perception. 5-hydroxytryptamine, the metabolite of tryptophan, play a kay role in regulating emotion by activating its receptor, but the

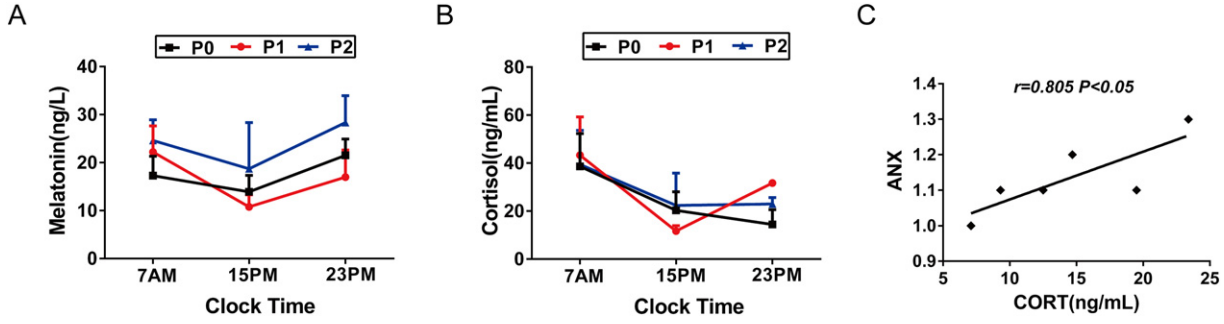


Fig. 6. Correlation between cortisol levels and psychological status. (A) General tendency of melatonin secretion by four crewmembers at three different stages. (B) General tendency of cortisol secretion by four crewmembers at three different stages. (C) High anxiety score demonstrated statistically significant associations with elevated cortisol level at P0 stage at 23:00 pm. Data is expressed as Mean ± SD, correlations were analyzed with Pearson's correlation coefficient. Note. ANX, anxiety; CORT, cortisol.

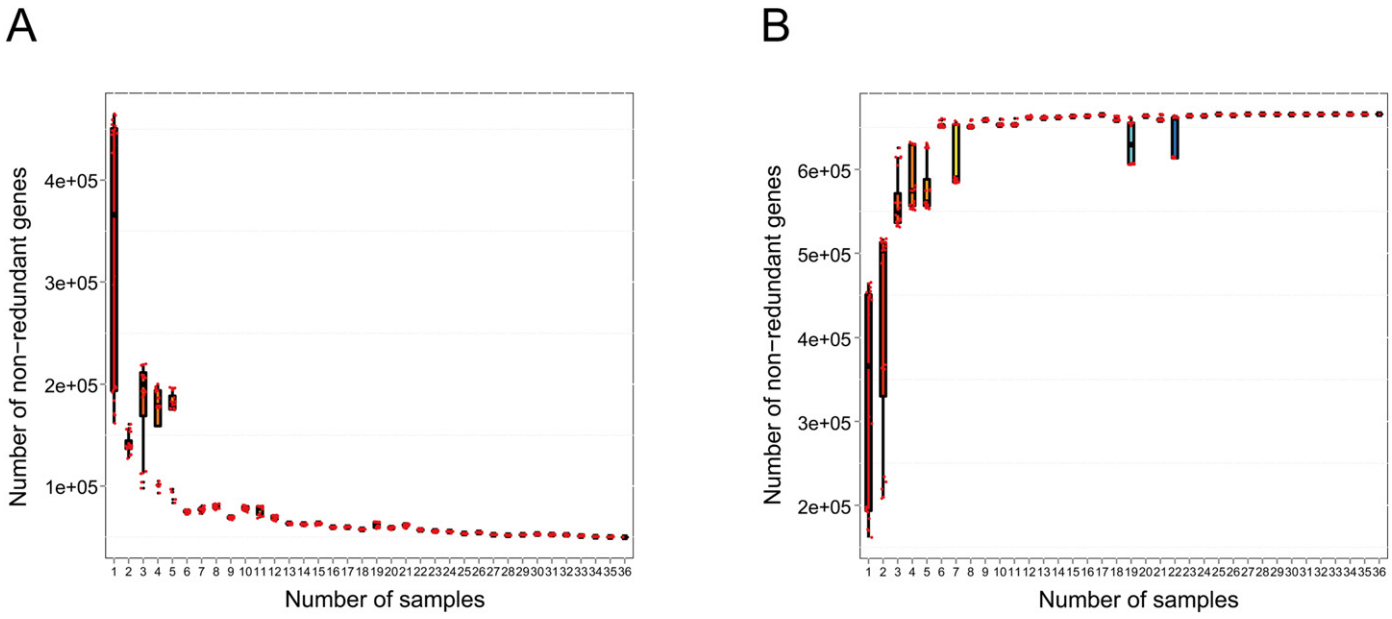


Fig. 7. Sample gene rarefaction curve. The rarefaction curves of Core (A) and Pan (B) genes. The abscissa represents the number of samples extracted, and the ordinate represents the number of genes in the sample combinations extracted.

mechanism is still undefined. When crewmembers entered to the stage lacking natural light transformation perception, they have psychological swings as above results showed. In addition, Li et al. reported a potential positive correlation between relative abundance of *Prevotella* and positive emotions in 2016 (Li et al., 2016). We guesses that metabolism and physiological activities is mobilized to stabilize emotion, such as intestinal microorganisms. Tryptophan metabolism may be one of the ways, and may also the reason why the relative abundance of *Prevotella* decreased at P1 stage.

4. Conclusions

To sum up, we can draw the conclusion that natural light and perception in an isolated environment has a beneficial on stabilize human

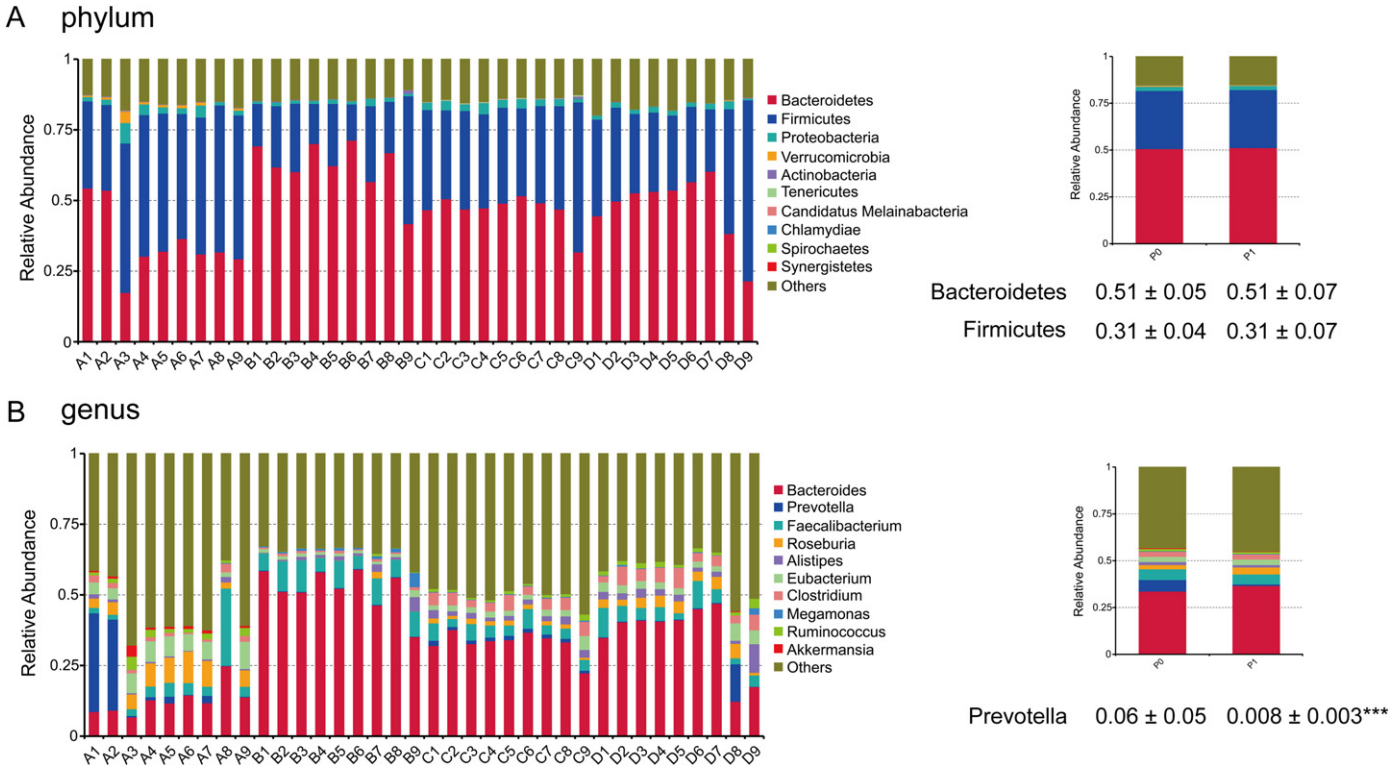


Fig. 8. The composition of intestinal microbiota in different isolated light environment. (A) Relative abundance of the top 10 phyla in each sample (left) and the relative abundance of top 10 phyla in each stage (right). The data in phylum level were analyzed by unpaired *t*-test. (B) Relative abundance of the top 10 genus in each sample (left) and the relative abundance of top 10 genus in each stage (right). The data in genus level were analyzed by unpaired *t*-test to obtain the significant different bacteria. Note. $^{***}P < 0.001$; data is expressed as Mean ± SD.

positive emotion, reduce the probability of emotional problems such as anxiety and depression, and also has a beneficial on hormone rhythmic secretion. Lacking natural light and perception in an isolated environment may have impact on human metabolism, the composition of intestinal microorganisms, as well as the risk of anxiety and depression, eventually influent on psychological and physiological health. These results can be used as a better understanding for the maintenance of human physical and mental health during in isolated environment. For example, natural light simulation in isolated environment can be designed to allow people to have a relatively normal natural light perception and also get natural light exposure.

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Abbreviations

SOM	somatization
O-C	obsessive-compulsive
I-S	interpersonal sensitivity
DEP	depression
ANX	anxiety
HOS	hostility
PHOB	phobic anxiety
PAR	paranoid ideation
PSY	psychoticism
SCL-90	symptom checklist 90
LP1	Lunar Palace 1
LC-MS	liquid chromatography–tandem mass spectrometric
PCA	principal component analysis
5-HTP	5-hydroxytryptophan

Declaration of competing interest

Chen Meng, Wei Wang and Zikai Hao performed the experiments, wrote the paper, and analyzed the data and prepared figures. Hong Liu conceived and designed the experiments, wrote the paper and prepared figures. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

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.

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