Gastroenterology Report, 2025, **13**, goaf031
https://doi.org/10.1093/gastro/goaf031
Original Article

Original Article

Long-term exposure to constant light disrupts intestinal stem cells through sympathoexcitation-induced Wnt5a signaling inhibition

Yu-Wan Wang[1,†], Qin-Yao Li[1,†], Ling-Feng Liu[2,†], Xing Tan[3], Wen Wang[3], Jia-Cen Sun[3,*] and Wei-Zhong Wang[1,*]

[1]Key Laboratory of Medical Electrophysiology of Ministry of Education, Medical Electrophysiology Key Lab of Sichuan Province (Collaborative Innovation Center for Prevention of Cardiovascular Diseases), Institute of Cardiovascular Research, Southwest Medical University, Luzhou, Sichuan, P. R. China
[2]School of Medicine, Shanghai University, Shanghai, P. R. China
[3]Naval Medical Center, Naval Medical University, Shanghai, P. R. China

*Corresponding authors. Wei-Zhong Wang, Southwest Medical University, No.1, Section 1, Xianglin Road, Longmatan District, Luzhou, Sichuan 646099, China. Tel: +86-18721297820; Email: weizhongwang@smmu.edu.cn; Jia-Cen Sun, Naval Medical Center, Naval Medical University (Second Military Medical University), 880 Xiangyin Road, Shanghai 200433, China. Tel: +86-19821238931; Email: sunjiacen1992@smmu.edu.cn

†Yu-Wan Wang, Qin-Yao Li, and Ling-Feng Liu contributed equally to this paper.

Abstract

Background: Long-term exposure to constant light is becoming a prevalent lifestyle that is associated with irritable bowel syndrome (IBS), a chronic functional gastrointestinal disorder. Intestinal stem cells (ISCs) are an important population of cells that maintain homeostasis and function of intestinal tissues. The purpose of this study was to identify the effects of long-term constant light exposure on gastrointestinal function and the potential mechanisms of sympathetic activity on ISC.

Methods: Rats housed in a 24 h constant light chamber for 4 weeks were used as the constant light exposure animal model. Hematoxylin-eosin staining and immunohistochemical examination were used to determine the pathological changes of the intestine. Propranolol (*ARs* inhibitor; 40 mg/kg/day), metoprolol (*ADRβ1* inhibitor; 50 mg/kg/day), and Box5 (*Wnt5a* inhibitor; 2 μg/day) were used to examine the effect of sympathoexcitation and Wnt signaling pathway on constant light-induced gastrointestinal disorders.

Results: We found that 4 weeks of constant light exposure in rats resulted in a decrease in the number of ISC and an increase in sympathetic activity. Intestinal β1-adrenoceptor expression and reactive oxygen species (ROS) were significantly increased, but *Wnt5a* expression decreased in the continuous light-exposed rats. Similarly, we found that administration of the β1-adrenoceptor antagonist metoprolol for 4 weeks attenuated the effects of continuous light exposure on the intestine, which was rescued by the reintroduction of *Wnt5a*.

Conclusion: Taken together, these data indicate that sympathoexcitation is critical for disruption of ISC under constant light exposure, suggesting that targeting β1-adrenoceptor/oxidative stress/*Wnt5a* axis may be a potential strategy for ISC disruption induced by prolonged sustained light exposure, providing a new direction for IBS treatment.

Keywords: constant light; sympathoexcitation; IBS; intestinal stem cells; ROS; Wnt5a

Introduction

Long-term exposure to constant light is a prevalent modern lifestyle under which individuals are more likely to suffer from gastrointestinal disorders [1–3]. Irritable bowel syndrome (IBS) is considered more prevalent among functional gastrointestinal disorders in terms of symptoms such as increased intestinal permeability and visceral sensitivity [4–7]. However, the correlation between constant light exposure and IBS remains insufficient, and the pathophysiology and underling mechanisms are still unambiguous.

Gut homeostasis mainly depends on the rapid renewing of intestinal epithelium, which requires a tight balance between intestinal stem cell (ISC) proliferation and differentiation [8, 9]. Emerging evidences suggested that IBS was closely associated with autonomic neuropathy especially sympathetic overactivation [10, 11]. Plenty of studies have confirmed that autonomic nerves could promote cell proliferation in the intestine by regulating ISC [12–14]. Sympathetic nerves reach to the level of enteric myofibroblasts and ISC, and adrenergic receptors (*ARs*) were expressed on these cells [14, 15]. Studies have proved abnormal sympathetic activation induced by constant exposure to light [16, 17]; however, to date, the regulatory functions and molecular mechanisms of sympathetic activation involved in the occurrence and development of IBS remain poorly understood. Here in this

Received: 12 April 2024. **Revised:** 04 October 2024. **Accepted:** 27 November 2024

study, we proposed a theory that constant exposure to light could cause ISC injury and IBS by enhancing sympathetic activity.

Wnt signaling pathway is critical in promoting ISC proliferation and thus intestinal epithelium regeneration [18, 19]. While in acetaminophen-injured livers, β-adrenoceptor agonist isoproterenol could totally activate Wnt signaling [20], suggesting a potential regulation of sympathetic activity on Wnt signaling pathway. Notably, sympathetic overactivation always stimulated excess reactive oxidative species (ROS), which has been proved to be able to trigger Wnt signaling pathway. However, the critical role of Wnt signaling pathway affordable for the sympathetic inhibition on ISC under constant exposure to light is still unclear.

In the current study, we mainly focused on the ISC injury caused by long-term exposure to constant light and explored the effect of sympathetic activity on Wnt signaling pathway. A 24-h constant light phase rat model was constructed, and intestinal physiological functions, intestinal pathological changes, changes in ISC and ROS-Wnt signaling pathway of this model were measured. Moreover, we determined the sympathetic effect on ISC by oral gavage of *ARs* inhibitor propranolol and metoprolol.

Materials and methods

Animals

Male Sprague-Dawley rats (8–12 weeks, 200–220 g) were housed with controlled temperature and humidity and a 12:12 h light–dark (LD) cycle (8 am light on, 8 pm light off). All experimental protocols adhered to ethical guidelines and were approved by the Animal Use and Care Committee of the Naval Medical University (IACUC protocol number: NMU-20210901).

Constant light exposure procedure

A time-controlled light animal rearing box (Shanghai Experimental Instrument Factory Co., Ltd, Shanghai, China) was used in this study, we utilized the white light-emitting diodes, with a wavelength of 465–485 nm, and the light intensity was adjusted to approximately 250–300 lux. Rats were acclimatized for 1 week and then housed in a 24-h constant light (LL) chamber with unrestricted food and water for 4 weeks to establish a constant light exposure model [21].

Gastrointestinal function testing

The rats were fasted within 12 h and then intragastrical administrated (ig) with 2 mL of a mixture of carbon powder and gum arabic, and then executed 30 min later with an overdose of pentobarbital sodium (200 mg/kg). The pylorus and cardia of rats were ligated with medical silk threads, and then the gastric emptying rate and small intestinal propulsion rate of rats were measured separately.

The whole stomach was removed and wiped dry, then weighed and recognized as the total gastric mass. Next, we cut along the greater curvature of the stomach with scissors, and washed it with saline to remove the contents. We weighed it which was recognized as the net gastric mass. The gastric emptying rate was calculated according to the following formula: [1 − (total gastric mass − net gastric mass)/gastric mass] × 100% [22].

The jejunum was clipped, and the distance from the pylorus to the advancement front of the carbon powder paste and its full length were measured, and the small intestine propulsion rate was calculated according to the following formula: length of carbon powder paste/full length of small bowel × 100% [23].

Evaluation of visceral sensitivity

The method for measuring evaluation of visceral sensitivity was based on the previous study [24]. Briefly, before the graded colonic distension test was performed, rats were forbidden from food intake but not from water for 12 h. A polyethylene balloon (about 4 cm) was inserted into the distal colon from the anus, and the end of the catheter was fixed at the root of the rat's tail with adhesive tape. The experiment was performed after 20 min according to the colonic distension pressures of 20, 40, and 60 mmHg, which lasted for 20 s each time, with a 4-min interval. Each pressure was measured three times, and the average of the three scores was taken. During each inflation period, a standard abdominal withdrawal score (AWR) was used to score the rats: 1, no obvious behavioral changes; 2, the rats' abdominal muscles contracted slightly but did not lift the abdomen off the platform; 3, the rats' abdominal muscles contracted markedly and the abdomen was lifted off the platform; and 4, the abdominal muscles contracted severely, the body was arched, and the pelvic structure was lifted off the platform.

Hematoxylin-eosin staining and immunohistochemical examination

The procedure of hematoxylin-eosin (HE) staining and immunohistochemical (IHC) examination was carried out as previously described [15]. After rats were perfused through the aorta with 0.9% NaCl solution (NS), jejunums were removed and refixed in 4% paraformaldehyde overnight at 4 °C, cut into 5-μm sections, and stained with HE.

We measured the expression of Olfm4, β1 adrenergic receptor (ADRB1), β2 adrenergic receptor (ADRB2), β3 adrenergic receptor (ADRB3), and tyrosine hydroxylase (TH) using IHC staining. Briefly, paraffin sections were deparaffinized, dehydrated, rehydrated, and repaired with microwave antigen, and the sections were blocked with 3% H_2O_2. Sections were then incubated overnight at 4°C with specific primary antibodies *Olfm4* (1:300; Affinity, China), ADRB1 (1:300; ZENBIO, China), ADRB2 (1:100; RecordBio, China), ADRB3 (1:200; Bioss, China) and TH (1:600; CST, USA), and then stained with HRP-conjugated secondary antibody. After sections were made, photographs were taken using the microscope (Leica, Germany). For each intestinal tissue section sample, we randomly selected four crypts, counted the positive cells in each crypt, and finally calculated the mean for statistical analysis.

Total RNA extraction and quantitative PCR

Total RNA from jejunum tissues was extracted with TransZol UP reagent (TransGen Biotech, China), and then RNA was reverse-transcribed into cDNA using a reverse transcription kit (TransGen Biotech), and the cDNA was amplified using SYBR Green qPCR Mix (TransGen Biotech). The relative expression was calculated using the 2-ΔCt method and normalized to *GAPDH*. All primers used in the experiment are listed in Table 1.

Western blotting

Based on previous study [15], jejunums tissues were collected, and the protein concentration of the intestinal tissues was determined by BCA Protein Assay Kit (Beyotime, China), diluted with 5× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5× SDS-PAGE) uploading buffer (Solarbio, China) and PBS (Solarbio, China) and boiled. Proteins were separated by SDS-PAGE in the 10% gradient gel (EpiZyme, China) and transferred to a polyvinylidene fluoride (PVDF) (Sigma, USA). After transfer, the PVDF was incubated with 5% skimmed milk (EpiZyme) in TBST for 2 h at room temperature. The primary antibody anti-Nicotinamide Adenine Dinucleotide Phosphate-Oxidase 2(NOX2) (1:800; Abcam, USA) and anti-*GAPDH* (1:5,000; ZENBIO) was diluted to the appropriate concentration in TBST, and then the

Table 1. SybrGreen primer sets used in qPCR experiments for rat samples

Gene ID	Forward (5′-3′)	Reverse (5′-3′)
GAPDH	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT
ZO-1	CAAGCCAGTCCATTCTCAGAGTCAG	TCCATAGCATCAGTTTCGGGTTTCC
Olfm4	AGCCGTCTTCTCCTCCTGTCC	GGCAGTCGTAGTCTCGGGTAATG
Wnt1	CTACTGGCACTGACCGCTCTG	GGTTCGTGGAGGAGGCTATGTTC
Wnt2	ATCTGGCTCTGGCTCCCTCTG	CCTGGCACATTGTCACACATCAC
Wnt2b	TCTGAAGCTGGAGTGCAAGTGTC	GTACGGCGGAAGTCTGAGAGTG
Wnt3	CTTTAAGCCACCCACGGAGAGG	CAGCAGATCGCAGCCATCAATG
Wnt3a	CAGCCTGACTTCCGCACCATC	TCCACCCAGCCACGAGACTC
Wnt4	ATACGCCATCTCTTCAGCAGGTG	CGGTCACAGCCACACTTCTCC
Wnt5a	CAGCCGAGAGACAGCCTTCAC	AGCCAGTCCCGAGGTAAGTCC
Wnt6	CTCCTCTACGCAGCCGATTCAC	AACAGGTCGCAGCCGCTAAG
Wnt7a	AAGGCAACCTGAGTGACTGTGG	CGTAGCGGATGTCGGCAGAG
Wnt8b	CCGACACCTTCCGTTCCATCTC	GGTCTTGTTCTCCAGGCAGTAGTC
Wnt9b	TGACGCCCACAACACCCATG	CTTCCAGCAGGTCCGCACAG
Wnt10a	AGTGCTTTCGCCTACGCCATAG	CATCGCAACCGCAAGCCTTC
Wnt11	CAACTACCTGCTTGACCTGGAGAG	GGGCGATGGTGTGACTGATGG

PVDF was incubated overnight at 4 °C. After that, the PVDF was washed by three times and then incubated with the secondary antibody for 2 h at room temperature. And the immunostaining bands were detected by an automatic chemiluminescence image analysis system. The band densities from Western blot were quantitated using ImageJ software (http://rsbweb.nih.gov/ij).

Drugs

The following drugs were used in the present study: propranolol (Pro; *ARs* inhibitor; ig, 40 mg/kg/day for 4 weeks) [25], metoprolol (Met; *ADRβ1* inhibitor; ig, 50 mg/kg/day for 4 weeks) [26], and Box5 (*Wnt5a* inhibitor; intraperitoneal injection [ip], 2 μg/day for 4 weeks) [27].

Enzyme-linked immunosorbent assay

Levels of norepinephrine (NE) and epinephrine (EPI) are commonly used to assess the functional state of sympathetic nerves. NE and EPI are key hormones of the sympathetic nervous system, and by measuring their levels in plasma, it is possible to assess the degree of sympathetic excitation and functional state. This is important for the study of physiological functions, pharmacological mechanisms and pathological states of sympathetic nerves *in vivo*. Concentrations of NE and EPI in the plasma were quantified by enzyme-linked immunosorbent assay (ELISA) kits (AiFang biological, China).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism software. Data are expressed as mean ± SD. Comparisons between the control and experimental groups were made using the student's *t*-test. Parametric tests were used for normally distributed data and non-parametric tests for non-normally distributed data. Comparisons between the different groups were made using one-way or two-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

Results

Effects of constant light on gastrointestinal function

To investigate the effect of constant light on gastrointestinal function, gastric emptying rate, small bowel propulsion rate, and visceral sensory sensitivity were recorded. Gastric emptying rate (LD vs LL (57.10 ± 14.40)% vs (38.10 ± 8.735)%, $P < 0.05$; Figure 1A) and intestinal propulsion rate (LD vs LL (69.67 ± 10.10)% vs (52.24 ± 8.193)%, $P < 0.05$; Figure 1B) were decreased in LL group than in LD group, whereas AWR scores (LD vs LL, 1.333 ± 0.516 vs 3.500 ± 0.548, $P < 0.001$) were increased in LL group than in LD group (Figure 1C). Meanwhile, LL group was accompanied with intestinal lumen fluid (Figure 1D) and shorter intestinal length (LD vs LL (131.3 ± 3.974) cm vs (117.9 ± 7.892) cm, $P < 0.01$; Figure 1E and F) compared with LD group.

In terms of intestinal morphology, more significant changes were also observed in LL group rats compared with the LD group rats, as evidenced by thicker muscularis layer (LD vs LL (28.19 ± 4.377) μm vs (55.14 ± 4.224) μm, $P < 0.001$), less goblet cells (LD vs LL, 24.08 ± 3.028 vs 6.542 ± 1.907, $P < 0.001$), shortened villi (LD vs LL (592.4 ± 31.16) μm vs (386.5 ± 41.17) μm, $P < 0.001$) and crypts (LD vs LL (158.2 ± 18.64) μm vs (83.46 ± 11.78) μm, $P < 0.001$; Figure 1G and H), as well as irregular arraying of villi together with villus fragmentation, and increased differentiation of crypts.

The expression of *ZO-1* gene was reduced in the LL group than in the LD group (LD vs LL, 1.0 ± 0.5705 vs 0.3567 ± 0.1700, $P < 0.05$; Figure 1I). Taken together, this evidence suggested that constant light exposure could cause intestinal damage, thus inducing gastrointestinal disorders.

Effects of sympathoexcitation on ISCs by constant light

In the present study, we found that the number of ISC was significantly lower in the LL group than in the LD group (LD vs LL, 5.25 ± 1.061 vs 2.705 ± 0.7416, $P < 0.001$; Figure 2A and B). Meanwhile, mRNA expression of *Olfm4*, a marker of ISC, was lower in the LL group than in the LD group (LD vs LL, 1.0 ± 0.1879 vs 0.6700 ± 0.2777, $P < 0.05$; Figure 2C), suggesting that constant light exposure affected the ISC, which may induce intestinal damage. We found that plasma NE (LD vs LL (3.972 ± 0.4282) ng/mL vs (4.993 ± 0.6290) ng/mL, $P < 0.01$) and EPI (LD vs LL (8.487 ± 1.471) ng/mL vs (10.74 ± 0.8443) ng/mL, $P < 0.01$; Figure 2D) concentrations were markedly increased; meanwhile, the integrated optical density (IOD) of intestinal TH (LD vs LL, 2.465 ± 0.8760 vs 3.535 ± 0.4669, $P < 0.05$) was significantly elevated in the LL group than in the LD group (Figure 2E and F), suggesting sympathetic hyperactivity under the constant light exposure condition. Furthermore, the number of ISC was significantly increased after treatment with β-adrenoceptor blocker propranolol in the LL group (LL_{PBS} vs LL_{Pro}, 2.600 ± 0.548 vs 4.950 ± 0.818, $P < 0.001$; Figure 2G and H), and *Olfm4* gene expression was similarly increased in the LL

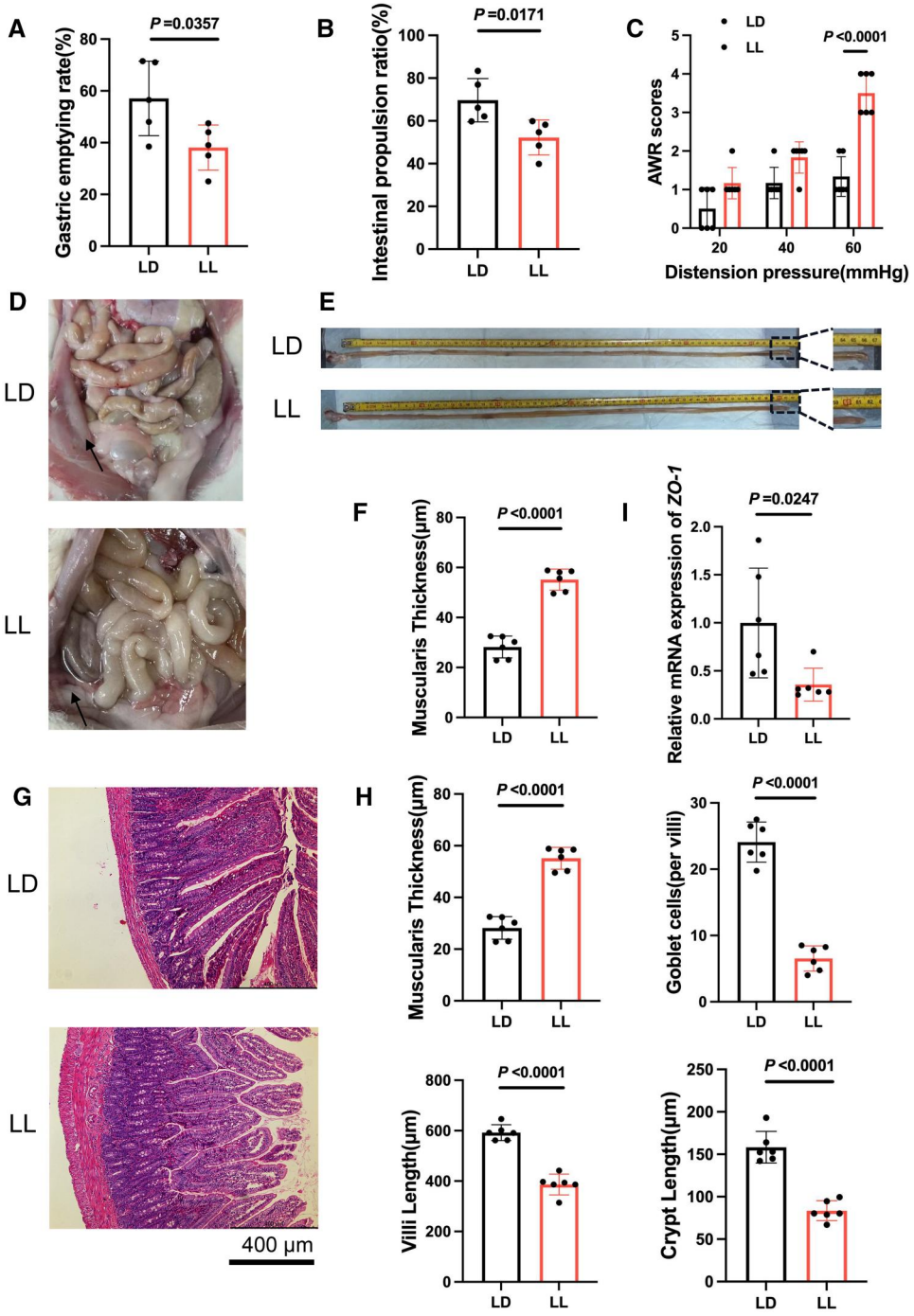

Figure 1. Constant light disrupts gastrointestinal function. (A, B) Statistical graphs of gastric emptying rate, intestinal propulsion rate in LD and LL groups. (C) AWR scores in LD and LL groups. (D) Representative images of abdominal cavity of LD and LL groups. (E, F) Representative images of intestine length and statistical graphs in LD and LL groups. (G, H) Representative HE-stained images of the intestine showing thickness of the muscle layer, number of goblet cells, length of villi, and length of crypts, with corresponding statistical graphs (scale bar = 400 μm). (I) Statistical graphs of relative *ZO-1* gene expression in LD and LL groups. $n = 5$–6 per group. LD = 12 h light: 12 h dark, LL = 24 h light, AWR = abdominal withdrawal score, HE = hematoxylin-eosin.

group (LL_{PBS} vs LL_{Pro}, 0.266 ± 0.126 vs 0.896 ± 0.182, $P < 0.05$; Figure 2I). This suggested that constant light may disrupts ISC through enhancing sympathetic activity.

Role of β1-adrenoceptors in constant light-induced ISC injury

To further investigate how sympathetic nerves affected ISC, it was found by IHC that intestinal *ADRβ1* was most increased in LL group rats than in LD group rats (Figure 3A). In addition, inhibition of *ADRβ1* by metoprolol in constant light exposure rats resulted in significant increases in the number of ISC (LL_{NS} vs LL_{Met}, 2.050 ± 0.512 vs 4.850 ± 0.576, $P < 0.001$; Figure 3B and C) and *Olfm4* mRNA expression (LL_{NS} vs LL_{Met}, 0.294 ± 0.168 vs 0.898 ± 0.112, $P < 0.05$; Figure 3D) compared with those treated with normal saline. Next, we determined whether ISC is affected by ROS, and the results showed that NOX2 expression was

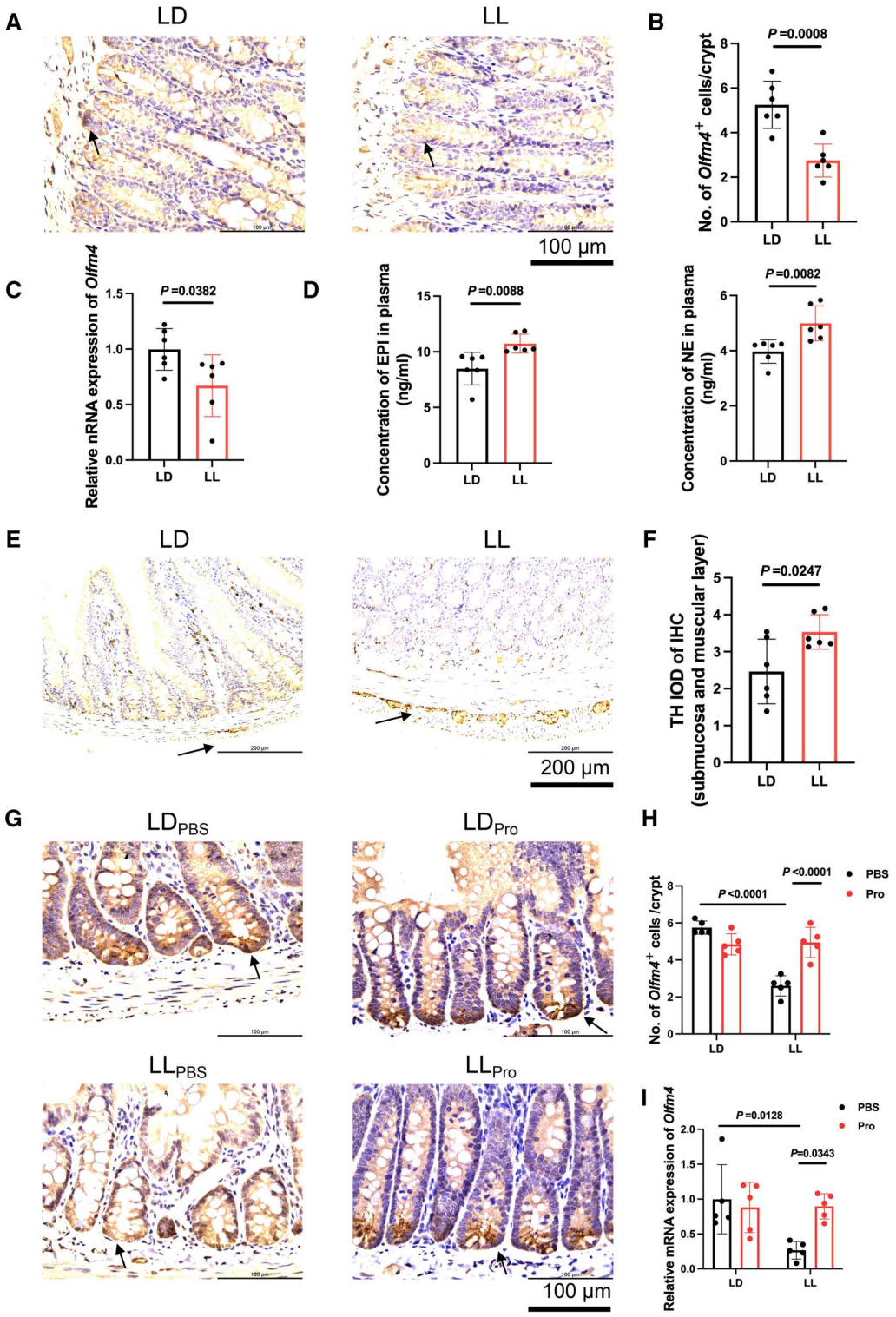

Figure 2. Sympathoexcitation is identified to be involved in ISC injury by constant light. (A, B) Representative IHC staining images of *Olfm4* positive cells (ISC) in each crypt and statistical graphs in the LD and LL groups (scale bar = 100 μm). (C) Statistical graphs of relative *Olfm4* gene expression in the two groups. (D) The concentration of NE and EPI in plasma of two groups. (E, F) Representative images of TH IOD in intestine submucosa and muscular layer in two groups with corresponding statistical graphs (scale bar = 200 μm). (G, H) Representative IHC staining images of *Olfm4* positive cells (ISC) in each crypt (scale bar = 100 μm) and statistical graphs. (I) Relative changes in *Olfm4* gene expression. $n = 5–6$ per group. LD = 12 h light: 12 h dark, LL = 24 h light, IHC = immunohistochemical, ISC = intestinal stem cells, NE = norepinephrine, EPI = epinephrine, TH = tyrosine hydroxylase, IOD = integrated optical density.

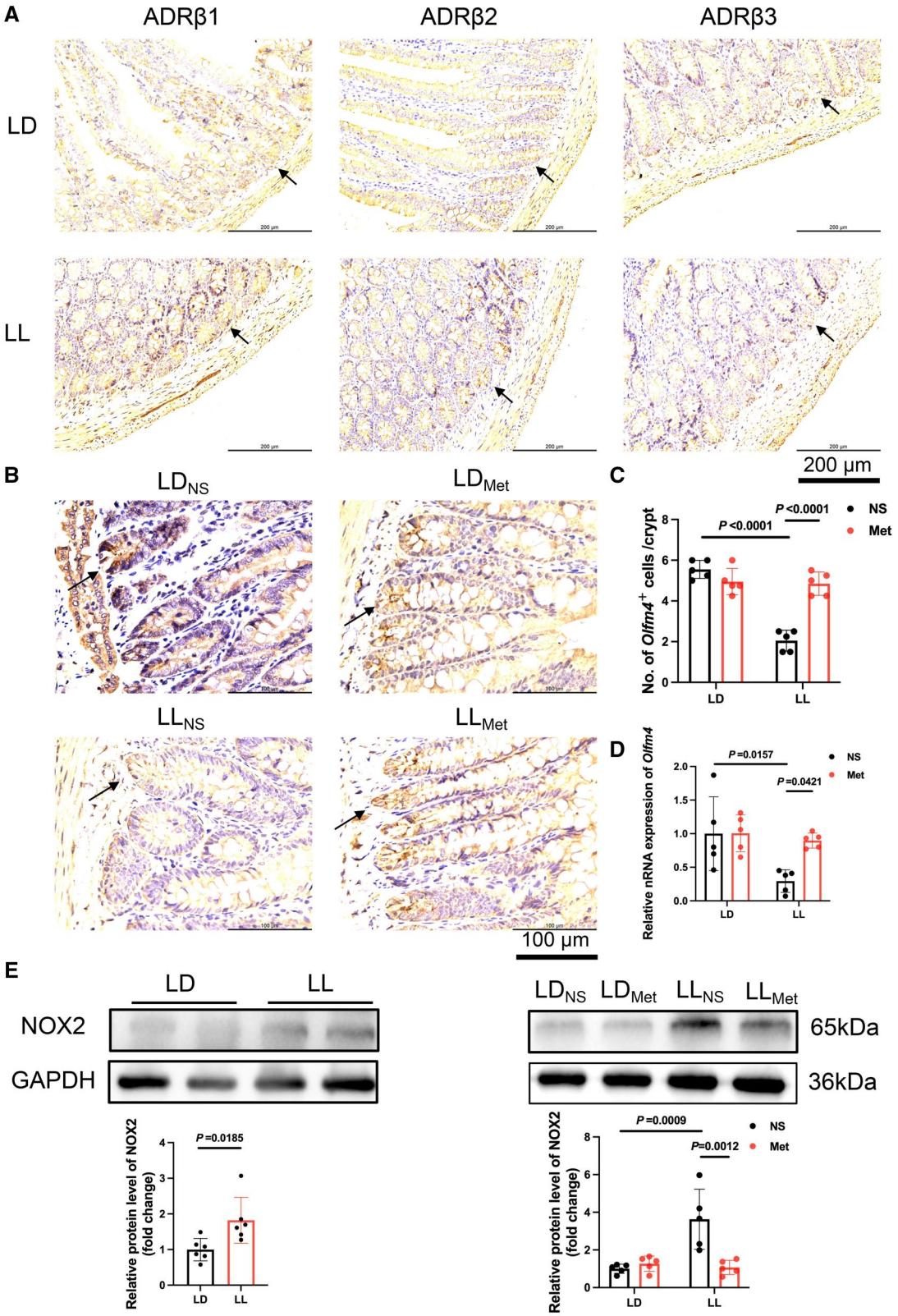

Figure 3. Constant light causes ISC injury through β1-adrenoreceptor. (A) Representative IHC staining images of ADRβ1, ADRβ2, and ADRβ3 in LD and LL groups (scale bar = 200 μm). (B, C) Representative IHC staining images of *Olfm4* positive cells in each crypt and statistical graphs in LD_{NS}, LD_{Met}, LL_{NS}, and LL_{Met} groups (scale bar = 100 μm). (D) Relative *Olfm4* gene expression in the four groups. (E) Expression of NOX2 was examined by Western blot and quantitated. $n = 5–6$ per group. LD = 12 h light: 12 h dark, LL = 24 h light, IHC = immunohistochemical, Met = metoprolol, NS = 0.9% NaCl solution.

significantly elevated in the LL group than in the LD group (LD vs LL, 1.000 ± 0.3136 vs 1.820 ± 0.6457, $P < 0.05$), while metoprolol significantly blunted this effect compared with rats treated with saline (LL_{NS} vs LL_{Met}, 3.628 ± 1.579 vs 1.072 ± 0.378, $P < 0.01$; Figure 3E).

Role of *Wnt5a* in sympathoexcitation-induced ISC injury by constant light

The expressions of 13 genes in the Wnt signaling pathway were examined by qPCR (Figure 4A), and we found that the expressions of *Wnt5a*, *Wnt8b*, and *Wnt10a* were downregulated in the LL group compared with the LD group. However, only *Wnt5a* mRNA expression was elevated after metoprolol treatment in the LL group (LL_{NS} vs LL_{Met}, 0.554 ± 0.118 vs 0.984 ± 0.251, $P < 0.05$; Figure 4B).

Compared with the LL_{Met} group, the LL_{Met} +Box5 group showed increased AWR scores (LL_{Met} vs LL_{Met} +Box5, 2.000 ± 0.707 vs 3.600 ± 0.548, $P < 0.01$; Figure 4C), decreased gastric emptying rate (LL_{Met} vs LL_{Met} +Box5 (58.120 ± 9.603)% vs (33.422 ± 7.408)%, $P < 0.01$; Figure 4D), decreased intestinal propulsion rate (LL_{Met} vs LL_{Met} +Box5 (63.120 ± 9.346)% vs (48.460 ± 7.028)%, $P < 0.05$; Figure 4E), thickened muscularis propria (LL_{Met} vs LL_{Met} +Box5 (35.088 ± 6.770) μm vs (54.070 ± 9.343) μm, $P < 0.01$), reduced number of goblet cells (LL_{Met} vs LL_{Met} +Box5 (22.600 ± 3.655) μm vs (11.316 ± 0.512) μm, $P < 0.05$), shortened length of villi (LL_{Met} vs LL_{Met} +Box5 (535.322 ± 18.955) μm vs (358.210 ± 50.912) μm, $P < 0.01$) and depth of crypts (LL_{Met} vs LL_{Met} +Box5 (146.138 ± 11.448) μm vs (80.208 ± 17.662) μm, $P < 0.05$; Figure 4F and G), reduced *ZO-1* mRNA expression (LL_{Met} vs LL_{Met} +Box5, 0.916 ± 0.105 vs 0.284 ± 0.147, $P < 0.05$; Figure 4H), decreased number of ISC (LL_{Met} vs LL_{Met} +Box5, 5.250 ± 0.661 vs 2.650 ± 0.518, $P < 0.001$; Figure 4I and J), and reduced *Olfm4* mRNA expression (LL_{Met} vs LL_{Met} +Box5, 0.872 ± 0.294 vs 0.384 ± 0.136, $P < 0.05$; Figure 4K). These experimental results suggested that *Wnt5a* was critical for sympathetic hyperactivity-induced intestinal damage caused by constant light exposure.

Effects of restoration of light rhythm on ISC function

To investigate whether removal of the light stimulator could restore gastrointestinal morphology, we brought rats exposed to 4 weeks constant light to normal 12 h light/12h dark phase. Here rats were divided into three groups as 4 weeks of continuous light exposure (LL), 6 weeks of continuous light exposure (LL_{6W}), and 2 weeks of light rhythm recovery after 4 weeks of continuous light exposure (LL_R). According to the results, it was found that the intestinal damage was enhanced in the LL_{6W} group compared with the LL group, as evidenced by decreased number of goblet cells (LL vs LL_{6W}, 15.4 ± 1.306 vs 10.25 ± 2.385, $P < 0.01$; Figure 5A and B), reduced *ZO-1* mRNA expression (LL vs LL_{6W}, 1.0 ± 0.1639 vs 0.2640 ± 0.2067, $P < 0.01$; Figure 5C), decreased number of ISC (LL vs LL_{6W}, 3.850 ± 0.3791 vs 1.900 ± 0.1369, $P < 0.001$; Figure 5D and E), and decreased expression of *Wnt5a* mRNA (LL vs LL_{6W}, 1.0 ± 0.4347 vs 0.2100 ± 0.06819, $P < 0.01$; Figure 5F). Interestingly, the above changes were partially ameliorated in the LL_R group. The above results suggested that light exposure might be an independent factor causing intestinal damage which was mediated by *Wnt5a* downregulation.

Discussion

Long-term exposure to constant light is prevalent in modern society where shift work, jet leg, and prolonged using electronics disturb circadian rhythm and increase the risk of IBS [28, 29]. However, our understanding of the potential mechanisms remains limited, which virtually constrains the efficient diagnosis and appropriate treatment. In the present work, we have shown that long-term exposure to constant light caused a significant disruption of ISC, which most likely led to IBS supported by the gastrointestinal function test and morphological detection. Importantly, our data demonstrated that sympathoexcitation was responsible for this effect. Mechanistically, *Wnt5a* signaling was markedly inhibited by β1-adrenoceptor-induced ROS activation after constant light exposure. These findings suggested that modulating sympathetic activity and oxidative stress might provide a new perspective to prevent and treat IBS via regulating the function of ISC.

Light is an important environmental signal triggering the circadian rhythm of individual activity. Long-term exposure to constant light disturbs the normal circadian regulation of central clock, affecting the peripheral rhythms [30]. It has been reported that circadian disruption was closely associated with the development of IBS [31], and light exposure was the upstream factor that governs the regular diurnal fluctuations of gut microbiota, while constant darkness led to the loss of the rhythmic oscillations in almost all parts of the intestine [32]. In addition, constant light exposure has long been recognized as a powerful behavioral stressor that could increase stress reactivity of hypothalamic–pituitary–adrenal (HPA) axis [33]. Both circadian disruption and stress are characterized by dysregulated autonomic nerve system activity, especially sympathetic activity [34, 35]. A recent study has demonstrated that stroke-induced gut permeability was mediated by the activation of the sympathetic nervous system [36], which serves as an important messenger mediating the crosstalk between other functional system and gastrointestinal tract. In the current study, we found that a 4-week constant light phase shift resulted in visceral hypersensitivity with a higher AWR score *in vivo*, smaller intestinal length, increased lumen fluid filling, and enhanced sympathetic activity ex vivo, compared with these in normal 12 h light/dark phase. Interestingly, we next found that a 4-week constant light with 2-week return to 12 h light/dark phase partly relieved these pathological changes, compared with rats exposed to 6-week constant light, suggesting that constant light might be an independent risk factor for gut homeostasis. However, the inability to clarify whether it is the light itself or the effects of circadian rhythm disruption caused by constant light exposure that brings about the effects is indeed a limitation of our study.

The effects of constant light exposure could be significantly blunted by administration of *ARs* inhibitor propranolol, suggesting a pivotal role of sympathetic participation in IBS under constant light exposure conditions. Subsequently, we revealed this sympathetic damage on gut homeostasis was mediated via β1-adrenoceptor, as its specific antagonist metoprolol similarly relieve the pathological condition of intestine. It has been reported that circadian disruption by light control caused disturbance of gastric vagal afferents, indicating that gastric vagal afferents are susceptible to disturbances in the light cycle [37], so we cannot exclude that parasympathetic inhibition was involved in constant light exposure-induced gastrointestinal disorder. Although these solid data suggested that sympathoexcitation might be harmful for gut homeostasis under the condition of constant light exposure, we cannot make a decision that sympathetic activation was a negative factor all the time, because studies have proved the protective effect of sympathetic activity in maintaining innate immunity of intestine [38, 39]. Moreover, gut homeostasis depends to a great extent on the presence of a balanced gut microbiota [40]. Nowadays, the "gut–brain axis" dysfunction

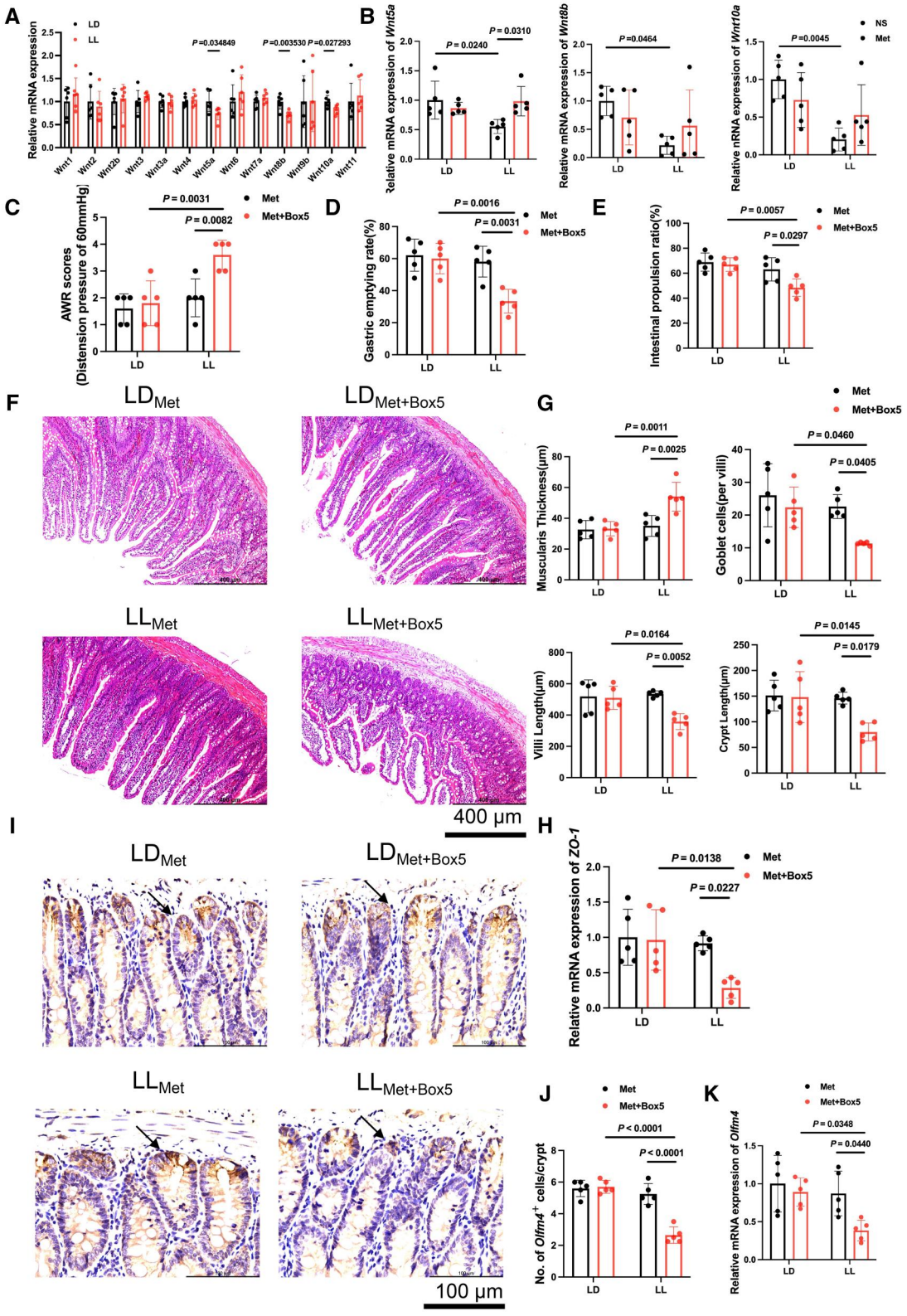

Figure 4. Sympathoexcitation inhibits *Wnt5a* signaling pathway by constant light. (A) Relative changes in the expression of 13 genes in the Wnt signaling pathway. (B) Relative *Wnt5a*, *Wnt8b*, and *Wnt10a* gene expression in LD_{NS}, LD_{Met}, LL_{NS}, and LL_{Met} groups. (C) AWR scores in LD_{Met}, $LD_{Met+Box5}$, LL_{Met}, and $LL_{Met+Box5}$ groups. (D, E) Statistical graphs of gastric emptying rate and intestinal propulsion rate in the four groups. (F, G) Representative HE-stained images of the intestine showing thickness of the muscle layer, number of goblet cells, length of villi, and length of crypts, with corresponding statistical plots (scale bar = 400 μm.). (H) Statistical graphs of relative changes in *ZO-1* gene expression in the four groups. (I, J) Representative IHC staining images of *Olfm4* positive cells in each crypt and statistical graphs in four groups and statistical graph (scale bar = 100 μm.). (K) Relative changes in *Olfm4* gene expression in the four groups. Here rats in metoprolol + saline group were abbreviated as Met. $n = 5–6$ per group. LD = 12 h light: 12 h dark, LL = 24 h light, IHC = immunohistochemical, Met = metoprolol, NS = 0.9% NaCl solution, AWR = abdominal withdrawal score.

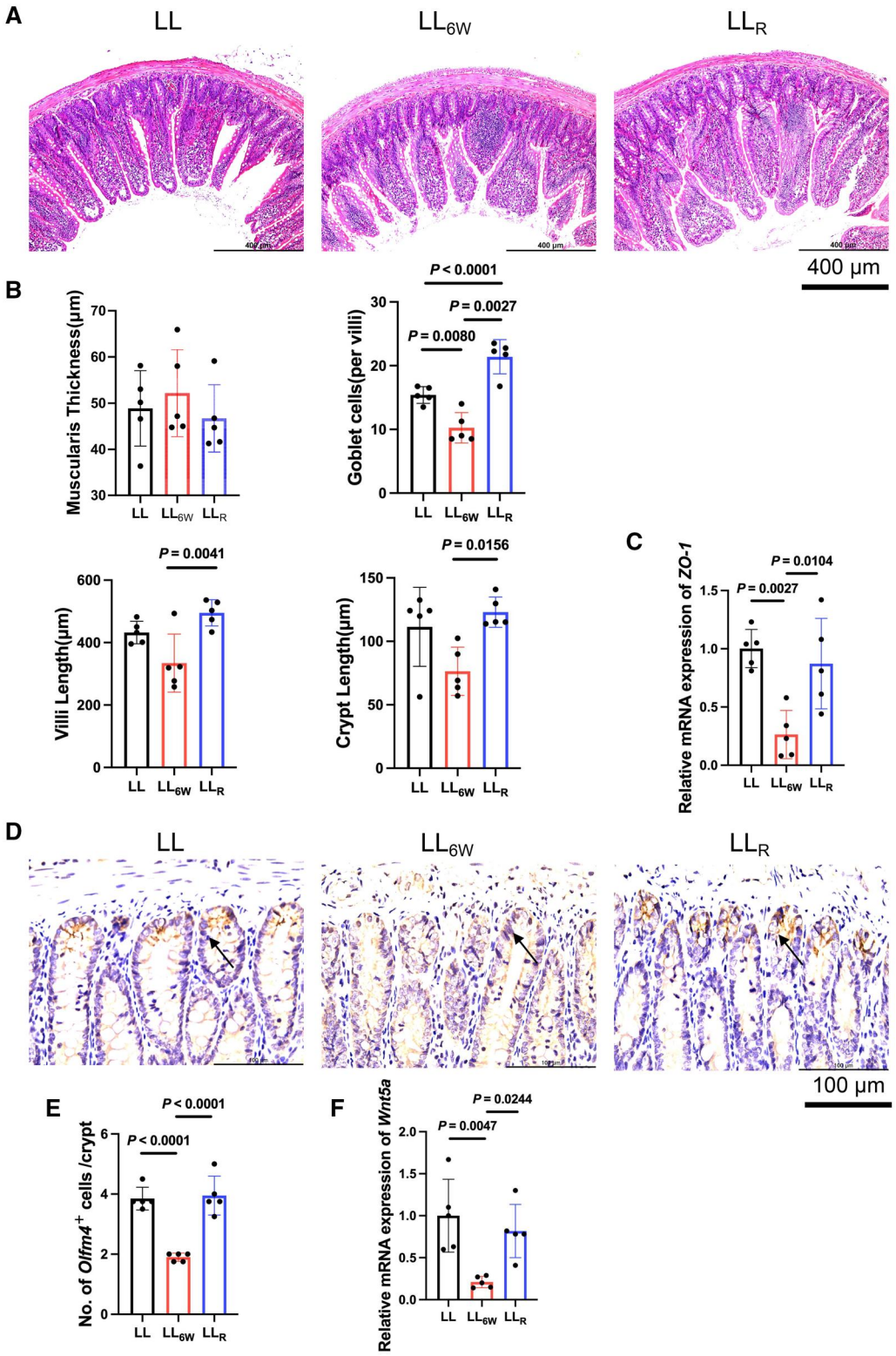

Figure 5. Restoration of light rhythm protected gastrointestinal function by constant light. (A, B) Representative HE-stained images of the intestine showing thickness of the muscle layer, number of goblet cells, length of villi, and length of crypts, with corresponding statistical plots (scale bar = 400 μm.). (C) Statistical graphs of relative *ZO-1* gene expression in LL, LL_{6W}, and LL_R groups. (D, E) Representative IHC staining images of *Olfm4* positive cells in each crypt and statistical graphs in the three groups and statistical graph (scale bar = 100 μm.). (F) Relative changes in *Wnt5a* gene expression in three groups. Rats with 2 weeks of light rhythm restoration after 4 weeks of constant light exposure were abbreviated as LL_R. $n = 5–6$ per group. AWR = abdominal withdrawal score, HE = hematoxylin-eosin, LL = 4 weeks of continuous light exposure, LL_{6W} = 6 weeks of continuous light exposure, LL_R = 2 weeks of light rhythm recovery after 4 weeks of continuous light exposure.

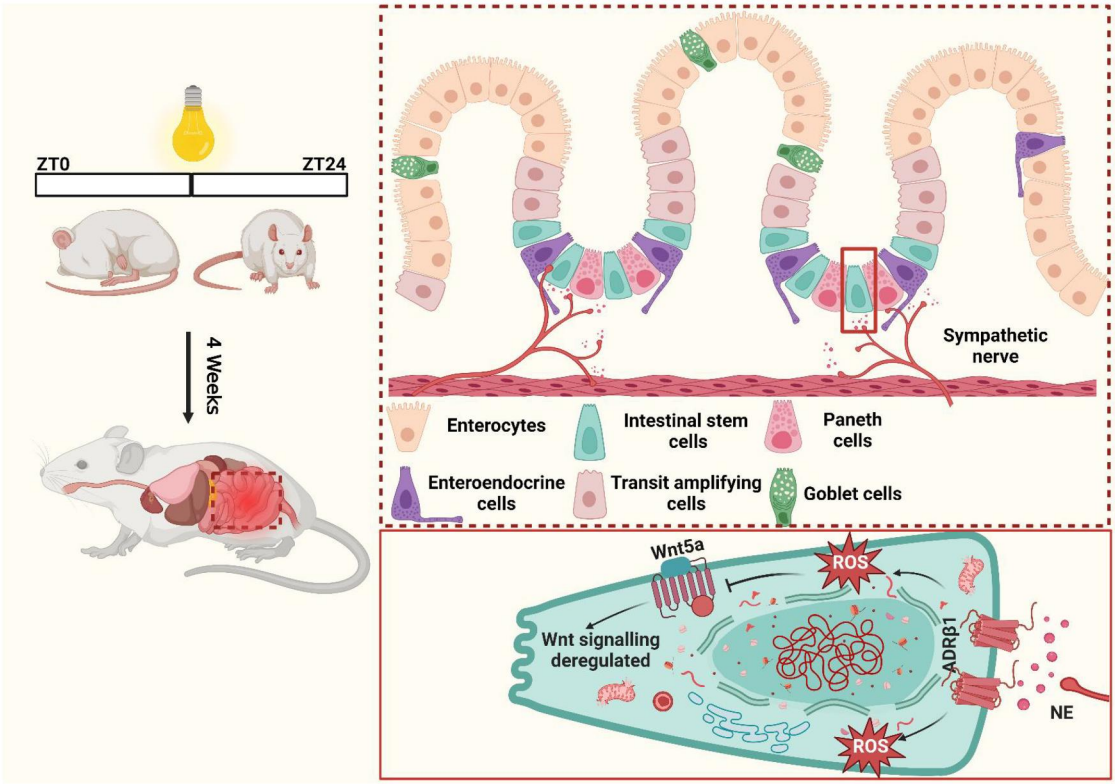

Figure 6. The schematic model of constant light on intestinal stem cells via sympathoexcitation-induced inhibition of *Wnt5a* signaling.

has been recognized responsible for many gastrointestinal diseases, sympathetic abnormality could disturb the homeostasis of gut microbiota and thus gastrointestinal function [41], while gut microbiota could control the extrinsic sympathetic activation through a gut–brain circuit [42]. As this gut microbiota-sympathetic related mechanism cannot be ruled out, we need further investigation to determine the effects of constant light exposure on gut microbiota and the relation to sympathetic overactivation-induced dysfunction of ISC.

Gut homeostasis is maintained mainly through the integrity of intestinal epithelial barrier, which is seriously dependent on the self-renewal and proliferation of ISC, while ISC injury could lead to a variety of gastrointestinal disorders [43, 44]. However, to date, the influence of constant light exposure on ISC and the pathological relevance of ISC remain elusive. Here, we revealed that constant light exposure caused a significant damage to ISC verified by lower *Olfm4* expression by immunohistochemistry measurement. Mechanistically, sympathetic overactivation inhibited *Wnt5a* signaling by eliciting ROS production. Here we cannot determine whether sympathetic nerve directly reached to intestinal crypts for small intestinal epithelial regeneration, nor the crosstalk between sympathetic nerve and intestinal microenvironment [45, 46], where biological information exchanges occur from the perspectives of Wnt signaling regulation [47, 48], which needs to be further confirmed.

Accumulating evidences underlie that Wnt signaling always plays an indispensable role in ISC function [49, 50]. The activity of Wnt signaling is regulated by a variety of stimulators such as amino acid, microbial metabolites, inflammatory cytokines, and oxidative stress [51–53]. Our findings provided insights into the mechanism by which sympathetic overactivation inhibited Wnt signaling pathway in response to constant light exposure from the perspective of ISC. In the present study, we had screened all Wnt signaling molecular affected by constant light exposure, and we found *Wnt5a* signaling was most sensitive to sympathetic activity. We further hypothesized that *Wnt5a* inhibition was associated with increased ROS production, which had been reported to be an important downstream effector of sympathetic activation [54], leading to different cellular biological damage. NADPH oxidase has been considered a mediator of the major source of ROS in activated macrophages and neutrophils in dysregulated gastrointestinal tract [55], and our results showed an increased expression of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase subtype NOX2. In order to determine the irreplaceable role of *Wnt5a* signaling in mediating sympathetic regulation of ISC function, we used *Wnt5a* specific antagonist Box5 to downregulate its signaling pathway, and the results could be reasonable to ask the question how ISC was injured. It has been demonstrated that Wnt/β-catenin signaling and the balance between Wnt and notch signaling activity are important to maintain the function of ISC [18, 56]. Thus, further studies are needed to elucidate the effects of sympathoexcitation on Wnt/β-catenin and Wnt/notch signaling in ISC.

Conclusions

Our findings revealed that long-term exposure to constant light caused a significant injury on ISC and gut homeostasis, which was mediated by sympathetic activity-induced *Wnt5a* signaling inhibition (Figure 6). These findings might help us to explore the valid strategy to prevent and treat the IBS to maintain the gut homeostasis under modern lifestyles such as constant light exposure.

Supplementary data

Supplementary data is available at *Gastroenterology Report* online.

Authors' contributions

All authors read and approved the final manuscript. W.Z.W., J.C.S., W.W., and X.T. contributed to the design of the experiment. Y.W.W. and Q.Y.L. performed the experiment. Q.Y.L. and L.F.L. analyzed the data. Y.W.W. wrote the manuscript with advice from Q.Y.L., L.F.L., W.W., X.T., J.C.S., and W.Z.W. All authors drafted the work or revised it critically and approved the final version of the manuscript. All authors have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This study was supported by the National Natural Science Foundation of China [Nos. 32100905 and 82270467], and China Postdoctoral Science Foundation [No. 2022M713841].

Conflicts of interest

All the authors report no relevant conflicts of interest for this article.

References

1. Wei L, Yue F, Xing L *et al.* Constant light exposure alters gut microbiota and promotes the progression of steatohepatitis in high fat diet rats. *Front Microbiol* 2020;**11**:1975.
2. Eum SY, Schurhoff N, Teglas T *et al.* Circadian disruption alters gut barrier integrity via a ss-catenin-MMP-related pathway. *Mol Cell Biochem* 2023;**478**:581–95.
3. Chen YD, Zhao RF, Zheng G, *et al.* The association between disruption of the circadian rhythm and aggravation of colitis in mice. *Gastroenterol Rep (Oxf)* 2022;**10**:goac028.
4. Sperber AD. Epidemiology and burden of irritable bowel syndrome: an international perspective. *Gastroenterol Clin North Am* 2021;**50**:489–503.
5. Ng SC, Shi HY, Hamidi N *et al.* Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* 2017;**390**:2769–78.
6. Hanning N, Edwinson AL, Ceuleers H *et al.* Intestinal barrier dysfunction in irritable bowel syndrome: a systematic review. *Therap Adv Gastroenterol* 2021;**14**:1756284821993586.
7. Hu L, Li G, Shu Y *et al.* Circadian dysregulation induces alterations of visceral sensitivity and the gut microbiota in light/dark phase shift mice. *Front Microbiol* 2022;**13**:935919.
8. Wang J, Zhao D, Lei Z *et al.* TRIM27 maintains gut homeostasis by promoting intestinal stem cell self-renewal. *Cell Mol Immunol* 2023;**20**:158–74.
9. Tian J, Li Y, Bao X *et al.* Glutamine boosts intestinal stem cell-mediated small intestinal epithelial development during early weaning: involvement of WNT signaling. *Stem Cell Rep* 2023;**18**:1451–67.
10. Hamrefors V, Fedorowski A, Ohlsson B. Susceptibility to diarrhea is related to hemodynamic markers of sympathetic activation in the general population. *Scand J Gastroenterol* 2019;**54**:1426–32.
11. Kwon S, Hsieh YS, Shin YK *et al.* Linalyl acetate prevents olmesartan-induced intestinal hypermotility mediated by interference of the sympathetic inhibitory pathway in hypertensive rat. *Biomed Pharmacother* 2018;**102**:362–8.
12. Kennedy MF, Tutton PJ, Barkla DH. Adrenergic factors involved in the control of crypt cell proliferation in jejunum and descending colon of mouse. *Clin Exp Pharmacol Physiol* 1983;**10**:577–86.
13. Davis EA, Zhou W, Dailey MJ. Evidence for a direct effect of the autonomic nervous system on intestinal epithelial stem cell proliferation. *Physiol Rep* 2018;**6**:e13745.
14. Davis EA, Dailey MJ. A direct effect of the autonomic nervous system on somatic stem cell proliferation? *Am J Physiol Regul Integr Comp Physiol* 2019;**316**:R1–R5.
15. Zeng H, Li H, Yue M *et al.* Isoprenaline protects intestinal stem cells from chemotherapy-induced damage. *Br J Pharmacol* 2020;**177**:687–700.
16. Jing JN, Wu ZT, Li ML *et al.* Constant light exerted detrimental cardiovascular effects through sympathetic hyperactivity in normal and heart failure rats. *Front Neurosci* 2020;**14**:248.
17. Chen M, Sun J, Chen TZ *et al.* Loss of nocturnal dipping pattern of skin sympathetic nerve activity during and following an extended-duration work shift in residents in training. *J Cardiol* 2021;**78**:509–16.
18. Zha JM, Li HS, Lin Q *et al.* Interleukin 22 expands transit-amplifying cells while depleting Lgr5(+) stem cells via inhibition of Wnt and notch signaling. *Cell Mol Gastroenterol Hepatol* 2019;**7**:255–74.
19. Farin HF, Jordens I, Mosa MH *et al.* Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. *Nature* 2016;**530**:340–3.
20. Soeda J, Mouralidarane A, Ray S *et al.* The beta-adrenoceptor agonist isoproterenol rescues acetaminophen-injured livers through increasing progenitor numbers by Wnt in mice. *Hepatology* 2014;**60**:1023–34.
21. Schilperoort M, van den Berg R, Coomans CP *et al.* Continuous light does not affect atherosclerosis in APOE3-Leiden.CETP mice. *J Biol Rhythms* 2020;**35**:598–611.
22. Jing FC, Zhang J, Feng C *et al.* Potential rat model of anxiety-like gastric hypersensitivity induced by sequential stress. *World J Gastroenterol* 2017;**23**:7594–608.
23. Zhou X, Chen Y, Ma X *et al.* Efficacy of Bacillus coagulans BC01 on loperamide hydrochloride-induced constipation model in Kunming mice. *Front Nutr* 2022;**9**:964257.
24. Chen Q, Zhang H, Sun CY *et al.* Evaluation of two laboratory model methods for diarrheal irritable bowel syndrome. *Mol Med* 2023;**29**:5.
25. Horinouchi T, Morishima S, Tanaka T *et al.* Different changes of plasma membrane beta-adrenoceptors in rat heart after chronic administration of propranolol, atenolol and bevantolol. *Life Sci* 2007;**81**:399–404.
26. Zhou Y, Xu M, Zhang Y *et al.* Effects of long-term application of metoprolol and propranolol in a rat model of smoking. *Clin Exp Pharmacol Physiol* 2014;**41**:708–15.
27. Wei X, Gong J, Ma J *et al.* Targeting the Dvl-1/beta-arrestin2/JNK3 interaction disrupts Wnt5a-JNK3 signaling and protects hippocampal CA1 neurons during cerebral ischemia reperfusion. *Neuropharmacology* 2018;**135**:11–21.
28. Liu H, Zou Y, Kan Y *et al.* Prevalence and influencing factors of irritable bowel syndrome in medical staff: a meta-analysis. *Dig Dis Sci* 2022;**67**:5019–28.
29. Sharma A, Goyal R. Long-term exposure to constant light induces dementia, oxidative stress and promotes aggregation of sub-pathological Abeta(42) in Wistar rats. *Pharmacol Biochem Behav* 2020;**192**:172892.

30. Koronowski KB, Sassone-Corsi P. Communicating clocks shape circadian homeostasis. *Science* 2021;**371**:eabd0951.
31. Nojkov B, Rubenstein JH, Chey WD *et al.* The impact of rotating shift work on the prevalence of irritable bowel syndrome in nurses. *Am J Gastroenterol* 2010;**105**:842–7.
32. Wu G, Tang W, He Y *et al.* Light exposure influences the diurnal oscillation of gut microbiota in mice. *Biochem Biophys Res Commun* 2018;**501**:16–23.
33. Goncharova N, Chigarova O, Oganyan T. Age-related and individual features of the HPA axis stress responsiveness under constant light in nonhuman primates. *Front Endocrinol (Lausanne)* 2022;**13**:1051882.
34. Liu K, Yang L, Wang G *et al.* Metabolic stress drives sympathetic neuropathy within the liver. *Cell Metab* 2021;**33**:666–75 e4.
35. Wang Y, Jiang W, Chen H *et al.* Sympathetic nervous system mediates cardiac remodeling after myocardial infarction in a circadian disruption model. *Front Cardiovasc Med* 2021;**8**:668387.
36. Prame Kumar K, McKay LD, Nguyen H *et al.* Sympathetic-mediated intestinal cell death contributes to gut barrier impairment after stroke. *Transl Stroke Res* 2023;**16**:280–98 doi: 10.1007/s12975-023-01211-y.
37. Kentish SJ, Christie S, Vincent A, *et al.* Disruption of the light cycle ablates diurnal rhythms in gastric vagal afferent mechanosensitivity. *Neurogastroenterol Motil* 2019;**31**:e13711.
38. Straub RH, Grum F, Strauch U *et al.* Anti-inflammatory role of sympathetic nerves in chronic intestinal inflammation. *Gut* 2008;**57**:911–21.
39. Willemze RA, Welting O, van Hamersveld P *et al.* Loss of intestinal sympathetic innervation elicits an innate immune driven colitis. *Mol Med* 2019;**25**:1.
40. Ulluwishewa D, Anderson RC, McNabb WC *et al.* Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* 2011;**141**:769–76.
41. Robles-Vera I, Toral M, de la Visitacion N *et al.* Changes to the gut microbiota induced by losartan contributes to its antihypertensive effects. *Br J Pharmacol* 2020;**177**:2006–23.
42. Muller PA, Schneeberger M, Matheis F *et al.* Microbiota modulate sympathetic neurons via a gut-brain circuit. *Nature* 2020; **583**:441–6.
43. Dotti I, Mora-Buch R, Ferrer-Picon E *et al.* Alterations in the epithelial stem cell compartment could contribute to permanent changes in the mucosa of patients with ulcerative colitis. *Gut* 2017;**66**:2069–79.
44. Du G, Xiong L, Li X *et al.* Peroxisome elevation induces stem cell differentiation and intestinal epithelial repair. *Dev Cell* 2020;**53**: 169–84 e11.
45. Straub RH, Stebner K, Harle P *et al.* Key role of the sympathetic microenvironment for the interplay of tumour necrosis factor and interleukin 6 in normal but not in inflamed mouse colon mucosa. *Gut* 2005;**54**:1098–106.
46. Leven P, Schneider R, Schneider L *et al.* Beta-adrenergic signaling triggers enteric glial reactivity and acute enteric gliosis during surgery. *J Neuroinflammation* 2023;**20**:255.
47. Colozza G, Lee H, Merenda A, *et al.* Intestinal Paneth cell differentiation relies on asymmetric regulation of Wnt signaling by Daam1/2. *Sci Adv* 2023;**9**:eadh9673.
48. Sailaja BS, He XC, Li L. The regulatory niche of intestinal stem cells. *J Physiol* 2016;**594**:4827–36.
49. Duan C, Wu J, Wang Z *et al.* Fucose promotes intestinal stem cell-mediated intestinal epithelial development through promoting Akkermansia-related propanoate metabolism. *Gut Microbes* 2023;**15**:2233149.
50. Jang H, Kim S, Kim H *et al.* Metformin protects the intestinal barrier by activating goblet cell maturation and epithelial proliferation in radiation-induced enteropathy. *Int J Mol Sci* 2022;**23**:5929.
51. Fang Z, Han X, Chen Y *et al.* Oxidative stress-triggered Wnt signaling perturbation characterizes the tipping point of lung adeno-to-squamous transdifferentiation. *Signal Transduct Target Ther* 2023;**8**:16.
52. Xing Y, Chen X, Cao Y *et al.* Expression of Wnt and Notch signaling pathways in inflammatory bowel disease treated with mesenchymal stem cell transplantation: evaluation in a rat model. *Stem Cell Res Ther* 2015;**6**:101.
53. Uchiyama K, Sakiyama T, Hasebe T *et al.* Butyrate and bioactive proteolytic form of Wnt-5a regulate colonic epithelial proliferation and spatial development. *Sci Rep* 2016;**6**:32094.
54. Hubens LE, Verloop WL, Joles JA *et al.* Ischemia and reactive oxygen species in sympathetic hyperactivity states: a vicious cycle that can be interrupted by renal denervation? *Curr Hypertens Rep* 2013;**15**:313–20.
55. Fournier BM, Parkos CA. The role of neutrophils during intestinal inflammation. *Mucosal Immunol* 2012;**5**:354–66.
56. Kaji I, Roland JT, Rathan-Kumar S, *et al.* Cell differentiation is disrupted by MYO5B loss through Wnt/Notch imbalance. *JCI Insight* 2021; **6**: e150416.

Gastroenterology Report, 2025, 13, –
https://doi.org/10.1093/gastro/goaf031
Original Article