

The LHA–DMV GABAergic Pathway Drives Constipation-predominant Irritable Bowel Syndrome via Modulating Colonic TRPV4

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Abstract

Background: Brain–gut axis dysfunction underlies constipation-predominant irritable bowel syndrome (IBS-C). The lateral hypothalamic area (LHA) and dorsal motor nucleus of the vagus (DMV) regulate autonomic and gastrointestinal functions. This study investigated the role and mechanism of the LHA–DMV GABAergic pathway in IBS-C pathogenesis.

Methods: IBS-C was induced in mice via chronic cold-water gavage. Fluoro-Gold retrograde tracing and immunofluorescence verified the LHA–DMV GABAergic projection and neuronal activity. Pharmacological rescue was performed by DMV microinjection of the GABAA receptor antagonist bicuculline (BIC). Chemogenetic activation of the LHA–DMV GABAergic circuit was achieved using viral transfection and clozapine N-oxide (CNO) treatment. Anxiety-like behaviors, intestinal transit, and visceral hypersensitivity were evaluated, and colonic TRPV4 expression was detected.

Results: Cold-water gavage successfully induced IBS-C-like phenotypes, including anxiety, delayed intestinal transit, and visceral hypersensitivity. A direct GABAergic projection from the LHA to the DMV was anatomically confirmed. DMV BIC administration alleviated stress-induced IBS-C symptoms. Chemogenetic activation of the LHA–DMV GABAergic pathway recapitulated IBS-C manifestations in normal mice, which were reversed by BIC. Colonic TRPV4 expression changes were consistent with behavioral and intestinal functional alterations.

Conclusions: The LHA–DMV GABAergic pathway critically modulates IBS-C progression. Circuit hyperactivation aggravates anxiety, intestinal dysmotility, and visceral hypersensitivity via DMV GABAA receptor signaling and subsequent colonic TRPV4 upregulation. This study identifies a novel brain–gut axis mechanism for IBS-C, offering potential neuromodulatory therapeutic targets.

Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by chronic abdominal discomfort and altered bowel habits, frequently accompanied by emotional disturbances such as anxiety¹. Driven by increased psychosocial stress, the global prevalence of IBS continues to rise, with Asian populations showing higher susceptibility to this disorder². As a major IBS subtype, constipation-predominant IBS (IBS-C) is clinically defined by persistent intestinal dysmotility, difficult defecation, and visceral hypersensitivity, substantially impairing patient quality of life and imposing a considerable socioeconomic burden. Owing to its multifactorial and incompletely elucidated pathogenesis, current IBS-C treatments only provide symptomatic relief without targeted disease-modifying therapies, resulting in high recurrence rates³. The etiology of IBS-C involves immune dysregulation, gut microbiota imbalance, visceral hypersensitivity, and defective brain–gut axis signaling⁴. Therefore, uncovering the core mechanisms underlying IBS-C brain–gut axis dysfunction is essential to advance novel therapeutic strategies.

As the core brainstem nucleus responsible for parasympathetic motor output to abdominal visceral organs, the dorsal motor nucleus of the vagus (DMV) constitutes the key dorsal vagal complex (DVC) together with the nucleus tractus solitaries (NTS)⁵⁻⁷. It is now well established that aberrant excitability of DMV neurons directly induces intestinal dysfunction and mediates IBS-related pathological phenotypes⁸. However, the upstream brain nuclei and specific neural circuit mechanisms that regulate DMV neuronal activity remain unclear; in particular, the central pathways mediating IBS-related visceral hypersensitivity await further investigation.

The lateral hypothalamic area (LHA) is a key hub integrating emotion, visceral sensation, and autonomic function, and is broadly involved in the central regulation of pain perception, anxiety, and gastrointestinal function⁹. GABAergic neurons in the LHA represent a core functional subtype that regulates negative emotions, visceral sensory transmission, and autonomic homeostasis. Through extensive descending projections, they participate in brain–gut axis modulation and serve as a key neuronal population linking central emotional disturbances to peripheral gastrointestinal dysfunction¹⁰. Although the evidence has confirmed the regulatory role of LHA activity in IBS-related pathological phenotypes¹¹, the precise downstream monosynaptic circuit basis remains poorly defined. Specifically, it remains unclear whether LHA GABAergic neurons regulate visceral hypersensitivity and intestinal dysfunction through direct monosynaptic innervation of downstream brainstem nuclei, and the underlying molecular regulatory mechanisms have not been elucidated. Accordingly, the specific downstream neural circuits and molecular mechanisms by which LHA GABAergic neurons modulate IBS pathogenesis urgently require systematic clarification.

Transient receptor potential vanilloid 4 (TRPV4) is a non-selective cation channel specifically expressed in colonic enteric glial cells, muscularis macrophages, and intestinal nerve fibers¹². TRPV4 mediates intestinal inflammatory pain and visceral hypersensitivity signaling, and its aberrant overexpression and activation represent a key peripheral mechanism driving visceral hypersensitivity in IBS¹³. In IBS model mice, TRPV4 expression is markedly upregulated in colonic tissue, and selective activation of TRPV4 directly elicits a visceral hypersensitivity phenotype¹⁴. Recent studies further indicate that neurotransmitter release and altered neuronal excitability within central circuits can regulate the expression and functional sensitivity of peripheral nociceptive TRPV4, thereby modulating visceral hypersensitivity¹⁵. However, whether the central LHA-DMV circuit contributes to the pathological progression of IBS by regulating TRPV4 expression remains unreported.

Integrating previous findings and our preliminary data, we hypothesize that hyperactivation of the LHA-DMV GABAergic neural circuit modulates peripheral colonic TRPV4 expression, thereby inducing visceral hypersensitivity, anxiety-like behaviors, and gastrointestinal dysmotility in IBS-C model mice. To validate this hypothesis, multiple experimental approaches were adopted in this study. Viral retrograde tracing combined with immunofluorescence staining was performed to verify the direct anatomical projection of LHA GABAergic neurons to the DMV; chemogenetic manipulation was applied to specifically activate the LHA-DMV GABAergic pathway, and comprehensive behavioral and functional assessments of anxiety,

visceral sensitivity, and intestinal motility were conducted; pharmacological blockade of DMV GABAA receptors was further used for rescue verification to clarify the core regulatory role of this circuit and the indispensable mediating function of TRPV4. Collectively, this study aims to elucidate the novel mechanism of the LHA-DMV GABAergic circuit in regulating IBS pathogenesis, enrich and supplement the theoretical system of brain–gut axis imbalance in functional gastrointestinal diseases, and provide novel experimental evidence and potential therapeutic targets for the treatment of IBS.

Materials and Methods

Animal Experiments

A total of 78 male C57BL/6J mice (6–8 weeks old, 18–22 g, SPF grade) were purchased from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. (license number: SCXK (Lu) 20220006). All mice had free access to food and water and were housed under a 12-h light/dark cycle at $24 \pm 1^\circ\text{C}$ and $45 \pm 5\%$ relative humidity. All experiments were approved by the Animal Ethics Committee of Qingdao University Medical College (Approval No. 20250901C57BL/610420260430120) and conducted in strict accordance with the Qingdao University Guidelines for the Care and Use of Laboratory Animals.

Reagents

The GABAA receptor antagonist bicuculline (BIC) and clozapine N-oxide (CNO) were purchased from GLPBIO. AAV2/9-mDlx-iCre-WPRE-pA (S0574-9-H5) and AAV2/2Retro Plus-hSyn-DIO-hM3D(Gq)-eGFP-WPRE-pA (S0260-2RP-H20) were obtained from Shanghai Taitool Bioscience Co., Ltd. Fluorogold (FG) was from Fluorochrome. Goat anti-GAD1/GAD67 antibody was from Novus Biologicals; rabbit anti-Fos antibody was from Cell Signaling Technology. Cy3-conjugated donkey anti-rabbit IgG and FITC-conjugated donkey anti-goat IgG were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. Mouse anti-HuC/D antibody was from Invitrogen; rabbit anti-TRPV4 antibody was from Immunoway. Alexa Fluor 488-conjugated goat anti-mouse IgG and Alexa Fluor 594-conjugated goat anti-rabbit IgG were obtained from Wuhan Sanying Biotechnology Co., Ltd.

Experimental Grouping

In Experiment 1, mice were randomly divided into two groups ($n = 6$ each): the normal group and the IBS group to verify IBS-like phenotypic changes induced by cold water gavage stress. In Experiment 2, FG was injected into the DMV of mice ($n = 6$); one week later, brains were collected by perfusion for fluorescence immunohistochemistry to identify LHA–DMV neural projections. In Experiment 3, after IBS-C model establishment, mice with DMV cannula implantation were divided into the normal group, IBS group, and BIC group to explore the therapeutic effect of GABAA receptor blockade on IBS-like symptoms ($n = 6$ each). In Experiment 4, normal mice receiving bilateral LHA injections of AAV2/9-mDlx-iCre-WPRE-pA and bilateral DMV injections of AAV2/2Retro Plus-hSyn-DIO-hM3D(Gq)-eGFP-WPRE-pA were randomly divided into two groups ($n = 6$ each): the normal saline (NS) group and the CNO group to confirm the regulatory role of the LHA-DMV GABAergic pathway. In Experiment 5, mice with identical

viral transfection were randomly assigned to four groups (n = 6 each): NS (i.p.) + NS (DMV) group, NS + BIC group, CNO + NS group, and CNO + BIC group to further validate the downstream mechanism of DMV GABAA receptor signaling.

Methods

IBS-C Model Establishment

Mice were administered 0.2 mL of 0–4°C cold water by gavage for 14 consecutive days¹⁶.

Fluoro-Gold Retrograde Tracing

Following our previously described method¹⁷, mice were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg). A volume of 200 nL of 2% FG was injected bilaterally into the DMV (AP –7.5 mm, ML ±0.35 mm, DV –4.5 mm from bregma). The needle was left in place for 10 min before withdrawal. Seven days later, mice were perfused and brains were collected for 15-µm cryosectioning.

Chemogenetics

A Cre-dependent designer receptor exclusively activated by designer drugs (DREADD) system was utilized for specific chemogenetic activation of LHA-originated DMV-projecting GABAergic neurons. AAV2/9-mDlx-iCre-WPRE-pA was bilaterally injected into the LHA (AP –1.34 mm, ML ±1.2 mm, DV –5.0 mm), and AAV2/2Retro Plus-hSyn-DIO-hM3D(Gq)-eGFP-WPRE-pA was bilaterally injected into the DMV. The needle was left in place for 10 min to prevent backflow. After a 3-week recovery, CNO (0.15 mg/kg) was administered intraperitoneally to activate the virus.

Intracerebral Cannula Implantation and Drug Administration

Mice were anesthetized and fixed in a stereotaxic frame. Stainless steel guide cannulas were implanted into the DMV according to the mouse brain atlas and secured with dental acrylic cement. After a 7-day recovery, a 10-cm polyethylene tube was connected to the injection cannula, and drug was infused continuously for 5 min. The injection cannula was left in place for an additional 2 min to allow complete diffusion.

Fecal Moisture Content (FMC)

Fresh fecal pellets were weighed immediately after expulsion, then dried in an oven at 60°C for 12 h. Fecal moisture content (%) = (wet weight – dry weight) / wet weight × 100%¹⁶.

First Black Stool Defecation Time (FDT)

After an 18-h fast, mice were gavaged with 0.3 mL of activated charcoal suspension. They were then placed in clean cages, and the time from charcoal administration to the first appearance of a black stool was recorded¹⁸.

Behavioral Tests

Open-field test (OFT): As previously described¹⁹, mice were placed in the peripheral zone and allowed to habituate for 30 s. Distance traveled and time spent in the center zone were recorded. The apparatus was cleaned with 75% ethanol between trials to eliminate olfactory cues and fecal residue.

Elevated plus maze (EPM): Following published protocols¹⁹, mice were placed on the central platform facing an open arm. After 30 s of habituation, the number of open-arm entries and time spent in the open arms were recorded. Cleaning was performed as above.

Marble burying test (MBT): A 47 cm × 25 cm × 30 cm cage was filled with 5 cm of bedding, and 20 glass marbles (1.5 cm diameter) were arranged in a 4 × 5 grid. After 30 min in a quiet environment, mice were returned to their home cages, and the number of marbles buried ($\geq 2/3$ of diameter) was counted¹¹.

Abdominal Withdrawal Reflex (AWR) Score

For colorectal distension (CRD), mice were fasted for 12 h and anesthetized with isoflurane. A lubricated deflated balloon was inserted into the colon 0.5 cm from the anal verge. Mice were placed in a 20 cm × 6 cm × 8 cm observation chamber and allowed to acclimate for 15 min. The balloon was then inflated to the designated pressure for 20 s, with a 4-min interval between inflations. Two blinded observers scored the AWR on a 0–4 scale: 0, no behavioral change; 1, immobility or simple head movement; 2, contraction of abdominal muscles with contact to the chamber; 3, abdominal muscle contraction without contact; 4, arching of the abdominal wall with or without body/pelvic elevation¹⁶.

Fluorescence Immunohistochemistry

Brain sections were washed, subjected to antigen retrieval, and blocked. They were incubated overnight at 4°C with rabbit anti-c-Fos (1:800), rabbit anti-TRPV4 (1:500), goat anti-GAD1/GAD67 (1:100), or mouse anti-HuC/D (1:100). On the following day, sections were incubated with corresponding secondary antibodies—FITC-conjugated donkey anti-goat IgG (1:100), Cy3-conjugated donkey anti-rabbit IgG (1:100), Alexa Fluor 488-conjugated goat anti-mouse IgG (1:500), or Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:500)—for 2 h at room temperature in the dark. Slides were mounted with DAPI-containing medium and observed under a microscope. Images of the LHA were analyzed using ImageJ by a blinded investigator. Sections of identical coordinates were used for each group. The number of GABA-positive cells per square millimeter and the percentage of double-labeled cell area (double-labeled area / total positive neuron area × 100) were calculated^{19,20}.

Western Blot

Western blot analysis was performed following standard protocols²¹. Proximal colon tissue was homogenized in RIPA lysis buffer (NCM Biotech) containing phosphatase and protease inhibitors on ice. Homogenates were centrifuged at 10,142 × g for 10 min, and supernatants were collected. Protein

concentration was determined using a BCA assay kit (Epipicyme). Equal amounts of protein (20 µg) were separated by 10% SDS-PAGE and transferred to 0.45 µm PVDF membranes (Solabio). Membranes were blocked with 5% non-fat milk in Tris-buffered saline containing Tween-20 (ZEN-Bio) for 2 h at room temperature, then incubated overnight at 4°C with primary antibodies: TRPV4 (1:1000; ZEN-Bio) and β-actin (1:7000; ZEN-Bio). After incubation with HRP-conjugated anti-rabbit secondary antibody (1:70,000; ZEN-Bio) for 2 h, bands were visualized using the ChemiScope 6200 system (Clinx Science Instruments, China) and quantified with ImageJ.

Histological Verification

At the end of the experiments, mice were perfused, and 25-µm frozen brain sections were prepared to verify injection sites. Data from animals with misplaced injections were excluded from analysis.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10.1.2. AWR scores are presented as median with 25th, 75th, 5th, and 95th percentiles; all other data are expressed as mean ± SEM. Comparisons between two independent groups were performed using Student's t-test. Multiple group comparisons were conducted by two-way ANOVA followed by Bonferroni/Dunn post hoc correction. $P < 0.05$ was considered statistically significant.

Results

Cold water gavage stress induces anxiety-like behaviors, constipation-like symptoms, and visceral hypersensitivity in mice

The timeline of Experiment 1 is shown in Fig. 1A. Following cold water gavage stress intervention, mice in the IBS-C model group exhibited significant behavioral abnormalities compared with the normal control group. In the OFT, the IBS group spent significantly less time and traveled a shorter distance in the center zone of the open field compared with the normal group ($P < 0.01$, $P < 0.05$; Fig. 1B). In the EPM, IBS mice exhibited significantly fewer open-arm entries and less time in the open arms than normal mice ($P < 0.05$, $P < 0.01$; Fig. 1C). The number of marbles buried was significantly increased in the IBS group ($P < 0.01$; Fig. 1D). Regarding intestinal function, the IBS group presented decreased FMC ($P < 0.01$; Fig. 1E), and a prolonged FDT ($P < 0.05$; Fig. 1F), indicating typical constipation-like symptoms. In addition, CRD assays showed that visceral hypersensitivity was significantly aggravated in IBS mice, as evidenced by elevated AWR scores under 40 mmHg, 60 mmHg, and 80 mmHg pressure stimulation ($P < 0.05$, $P < 0.01$; Fig. 1G). Fluorescence immunohistochemistry showed that the number of GABA-immunoreactive neurons in the LHA was significantly increased in the IBS group ($P < 0.01$; Fig. 1H), and the percentage of c-Fos/GABA co-labeled cells increased from $22.54 \pm 6.57\%$ to $36.39 \pm 6.65\%$ ($P < 0.01$; Fig. 1H).

GABA-immunoreactive neurons in the LHA project to the DMV

FG was injected into the DMV, with the injection site shown in Fig. 2A. Seven days later, FG-labeled cells were detected in the LHA. Among GABA-positive neurons, $41.85 \pm 4.51\%$ contained FG, and $31.74 \pm 3.91\%$ of FG-labeled neurons exhibited GABA immunoreactivity (Fig. 2B), indicating that a subset of GABA-immunoreactive neurons in the LHA projects to the DMV.

GABAA receptor antagonist in the DMV ameliorates IBS-like symptoms in mice

The experimental procedure for Experiment 3 is illustrated in Fig. 3A. Microinjection of the GABAA receptor antagonist BIC into the DMV significantly increased both the time spent and distance traveled in the center zone of the open field ($P < 0.05$; Fig. 3B), and increased the number of open-arm entries and time spent in the open arms compared with the IBS group ($P < 0.05$; Fig. 3C). The number of marbles buried was markedly reduced after BIC intervention ($P < 0.05$; Fig. 3D). In terms of intestinal dysfunction, DMV BIC microinjection effectively ameliorated constipation-like symptoms, including increased FMC and shortened FDT (both $P < 0.05$; Fig. 3E, 3F). AWR scores at 60 and 80 mmHg CRD pressures were significantly decreased after GABAA receptor blockade ($P < 0.05$; Fig. 3G). At the cellular and molecular levels, immunofluorescence staining showed that the co-localization ratio of TRPV4 and the ENS marker HuC/D decreased from $32.66 \pm 5.67\%$ to $25.85 \pm 4.26\%$ ($P < 0.05$; Fig. 3H). Consistently, western blot results verified that colonic TRPV4 protein expression was significantly downregulated following DMV GABAA receptor inhibition ($P < 0.05$; Fig. 3I).

Chemogenetic activation of the LHA-DMV GABAergic pathway induces IBS-like symptoms in normal mice

The experimental procedure for Experiment 4 is shown in Fig. 4A. The Cre-dependent chemogenetic viral vector AAV2/9-mDlx-iCre was injected into the LHA, and retrograde AAV2/2Retro Plus-hSyn-DIO-hM3D(Gq)-eGFP was delivered to the DMV to specifically label and activate LHA-originated DMV-projecting GABAergic neurons (Fig. 4B). Although no significant difference was found in central zone residence time in the OFT between the CNO and NS groups ($P > 0.05$; Fig. 4C), the central traveling distance was significantly reduced in the CNO group ($P < 0.05$; Fig. 4C). Meanwhile, chemogenetic activation decreased open-arm entries and open-arm residence time in the EPM test ($P < 0.05$; Figure 4D) and increased the number of buried marbles ($P < 0.05$; Fig. 4E), indicating aggravated anxiety-like behaviors. In addition, FMC was lower ($P < 0.01$; Fig. 4F), and the FDT was significantly prolonged ($P < 0.05$; Fig. 4G). Moreover, AWR scores at 40, 60, and 80 mmHg CRD pressures were significantly elevated in CNO-treated mice ($P < 0.05$; Fig. 4H), confirming that specific activation of the LHA-DMV GABAergic neural pathway is sufficient to induce visceral hypersensitivity and IBS-like syndromes in healthy mice.

GABAA receptor antagonist in the DMV reverses IBS-like symptoms induced by LHA-DMV GABAergic activation

The experimental procedure for Experiment 5 is shown in Fig. 5A. Viral injection was consistent with Experiment 4, and either BIC or NS was selectively microinjected into the DMV. Compared with the CNO+NS group, the CNO+BIC group showed no significant difference in time spent in the center zone of

the open field ($P>0.05$; Fig. 5B), but showed a significant increase in central traveling distance ($P<0.05$; Fig. 5B). The number of open-arm entries and time spent in the open arms were significantly increased ($P<0.01$, $P<0.05$; Fig. 5C). The number of marbles buried was significantly reduced ($P<0.05$; Fig. 5D). For intestinal function indicators, DMV BIC intervention significantly increased FMC and shortened the prolonged FDT (both $P<0.05$; Fig. 5E, 5F). Meanwhile, the elevated AWR scores at 60 mmHg and 80 mmHg in chemogenetically activated mice were markedly reversed by GABAA receptor antagonism ($P<0.05$; Fig. 5G). At the molecular level, the increased co-localization ratio of TRPV4 and HuC/D in the ENS was reduced from $29.23 \pm 5.38\%$ to $22.39 \pm 4.17\%$ ($P<0.05$; Fig. 5H), and the upregulated colonic TRPV4 protein expression was significantly downregulated in the CNO+BIC group ($P<0.05$; Fig. 5I). Collectively, these rescue experiments demonstrate that the LHA-DMV GABAergic pathway modulates IBS-like symptoms via DMV GABAA receptor/TRPV4 signaling.

Discussion

As a common functional gastrointestinal disorder, current clinical management of IBS focuses primarily on alleviating symptoms such as abdominal pain, diarrhea, and constipation, but overall therapeutic efficacy remains unsatisfactory¹⁹, underscoring the urgent need to explore its pathogenesis and develop novel treatment strategies. Establishing stable and reliable animal models is essential for investigating IBS mechanisms. Cold-water gavage stress has been widely used to generate constipation-predominant IBS-C models, which recapitulate core symptoms including visceral hypersensitivity and constipation, with advantages of simple operation and good reproducibility²². Our results demonstrate that mice subjected to cold-water gavage stress exhibited anxiety-like behavior, constipation-like symptoms, and visceral hypersensitivity, accompanied by significantly increased c-Fos expression in LHA GABAergic neurons, suggesting that LHA GABAergic neurons are persistently overactivated under chronic stress and may be closely implicated in stress-induced emotional disorders and intestinal functional dysfunction in IBS.

The LHA is a key center regulating feeding, energy metabolism, arousal, and autonomic nervous activity, and its role in gastrointestinal modulation has garnered increasing attention in recent years^{23, 24}. Classic neuroanatomical studies have established that the LHA, together with the paraventricular hypothalamic nucleus, provides direct input to medullary autonomic preganglionic neurons, including those located in the DMV²⁵. It is known that LHA neurons directly project to the DVC, which comprises the DMV and the NTS²⁶, and electrical stimulation of the LHA markedly alters spontaneous firing of DMV and NTS neurons, indicating that the LHA participates in the regulation of vagus nerve-mediated gastrointestinal physiological functions through descending neural pathways. However, most previous studies focused on the overall functional correlation between the LHA and gastrointestinal regulation, lacking direct anatomical and functional evidence for the specific neuronal subtype pathway. Specifically, the existence of a direct monosynaptic GABAergic projection from the LHA to the DMV and its exact functional role in IBS pathogenesis and visceral hypersensitivity remain unclear. In the present study, retrograde Fluoro-Gold tracing combined with immunofluorescence co-localization analysis systematically verified the

direct anatomical projection of LHA GABAergic neurons to the DMV, providing definitive structural evidence for the formation of the LHA-DMV GABAergic neural circuit.

The DMV serves as the core brainstem nucleus of parasympathetic preganglionic neurons, extensively innervating the gastrointestinal tract and accessory digestive organs, and is responsible for the central regulation of intestinal peristalsis, visceral sensation transduction, and gastrointestinal secretion²⁷. DMV neuronal activity is precisely modulated by abundant upstream synaptic inputs, among which GABAergic inhibitory signaling is the predominant regulatory pathway. GABAA receptors are ubiquitously and abundantly expressed in DMV neurons, constituting the core molecular basis for GABAergic inhibitory modulation²⁸. Evidence shows that DMV GABAA receptors receive continuous tonic inhibitory inputs from upstream central nuclei such as the NTS, which is essential for maintaining basal gastrointestinal tension and normal intestinal motility. Pharmacological blockade of DVC GABAA receptors via microinjection of BIC can significantly enhance DMV neuronal excitability, improve gastrointestinal motility, and promote digestive secretion²⁹. Consistent with these findings, our pharmacological intervention experiments further confirmed the critical regulatory role of DMV GABAA receptor signaling in IBS progression. Inhibition of DMV GABAA receptor activity effectively reversed anxiety-like behaviors, intestinal dysmotility, and visceral hypersensitivity in IBS model mice, demonstrating that excessive GABAergic inhibitory signaling in the DMV is a key pathogenic factor driving IBS-like symptoms.

In this study, specific chemogenetic activation of the LHA-DMV GABAergic circuit was sufficient to trigger comprehensive IBS-like pathological phenotypes, including anxiety-like behaviors, constipation-like intestinal dysmotility, and visceral hypersensitivity. More importantly, rescue experiments confirmed that blockade of DMV GABAA receptors significantly reversed the pathological abnormalities induced by LHA-DMV GABAergic hyperactivation. These results fully demonstrate that the LHA-DMV GABAergic pathway acts as a critical upstream central regulatory circuit in IBS pathogenesis, and its pro-IBS function is strictly dependent on downstream DMV GABAA receptor signaling.

In terms of peripheral brain–gut axis downstream mechanisms, TRPV4 has been identified as a key peripheral molecular sensor mediating intestinal visceral hypersensitivity. Clinical evidence has shown that TRPV4 channel sensitization in intestinal mucosal tissue is closely correlated with abdominal pain and bloating symptoms in IBS patients³⁰. Multiple peripheral active mediators including histamine and 5-HT can significantly enhance TRPV4 channel activity and protein stability, thereby amplifying intestinal visceral sensory responses and aggravating visceral hypersensitivity³¹. Endogenous TRPV4 agonists (e.g., 5,6-EET) are significantly elevated in the colonic tissue of IBS patients and positively correlate with abdominal pain and bloating scores, further supporting a central role of TRPV4 in IBS-related visceral hypersensitivity¹³. In the present study, we found that the colonic TRPV4 expression level was significantly upregulated in IBS model mice with overactivated LHA-DMV GABAergic signaling. In contrast, pharmacological inhibition of DMV GABAA receptors blocked central circuit hyperactivation and effectively downregulated colonic TRPV4 expression, accompanied by significant amelioration of IBS-like symptoms. These findings reveal a novel central-peripheral regulatory axis: the overactive LHA-

DMV GABAergic pathway sustains high colonic TRPV4 expression, thereby maintaining persistent visceral hypersensitivity and intestinal dysfunction in IBS. Combined with previous reports, we speculate that this peripheral regulatory process may be closely associated with downstream histamine/5-HT/TRPV4 signal sensitization, which further amplifies intestinal functional disorders.

Conclusion

The present study systematically demonstrates that the LHA-DMV GABAergic neural circuit serves as a critical upstream regulatory pathway for IBS pathogenesis. Hyperactivation of this circuit induces anxiety-like behaviors, constipation-like intestinal dysmotility, and visceral hypersensitivity in mice. Mechanistically, the pathological regulatory effect of this pathway is dependent on DMV GABA_A receptor-mediated central inhibitory signaling and is closely associated with the upregulation of peripheral colonic TRPV4 expression, which may be mediated by the downstream histamine/5-HT/TRPV4 signaling cascade. These findings reveal a novel central-peripheral integrated mechanism underlying IBS brain–gut axis dysfunction, providing innovative theoretical basis and potential therapeutic targets for the mechanism research and clinical intervention of IBS.

Declarations

Author Contributions

Conceptualization: Feifei Guo; Methodology: Tingting Hou, Yuyan Wang, Jia Su, Xiaoyu Zuo, Xiao Ma, Baoshuo Wu, Jiayang Sun; Formal analysis and investigation: Hua Liu; Writing - original draft preparation: Tingting Hou, Yuyan Wang; Writing - review and editing: Feifei Guo; Supervision, Funding Acquisition: Feifei Guo. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare no competing interests.

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Figures

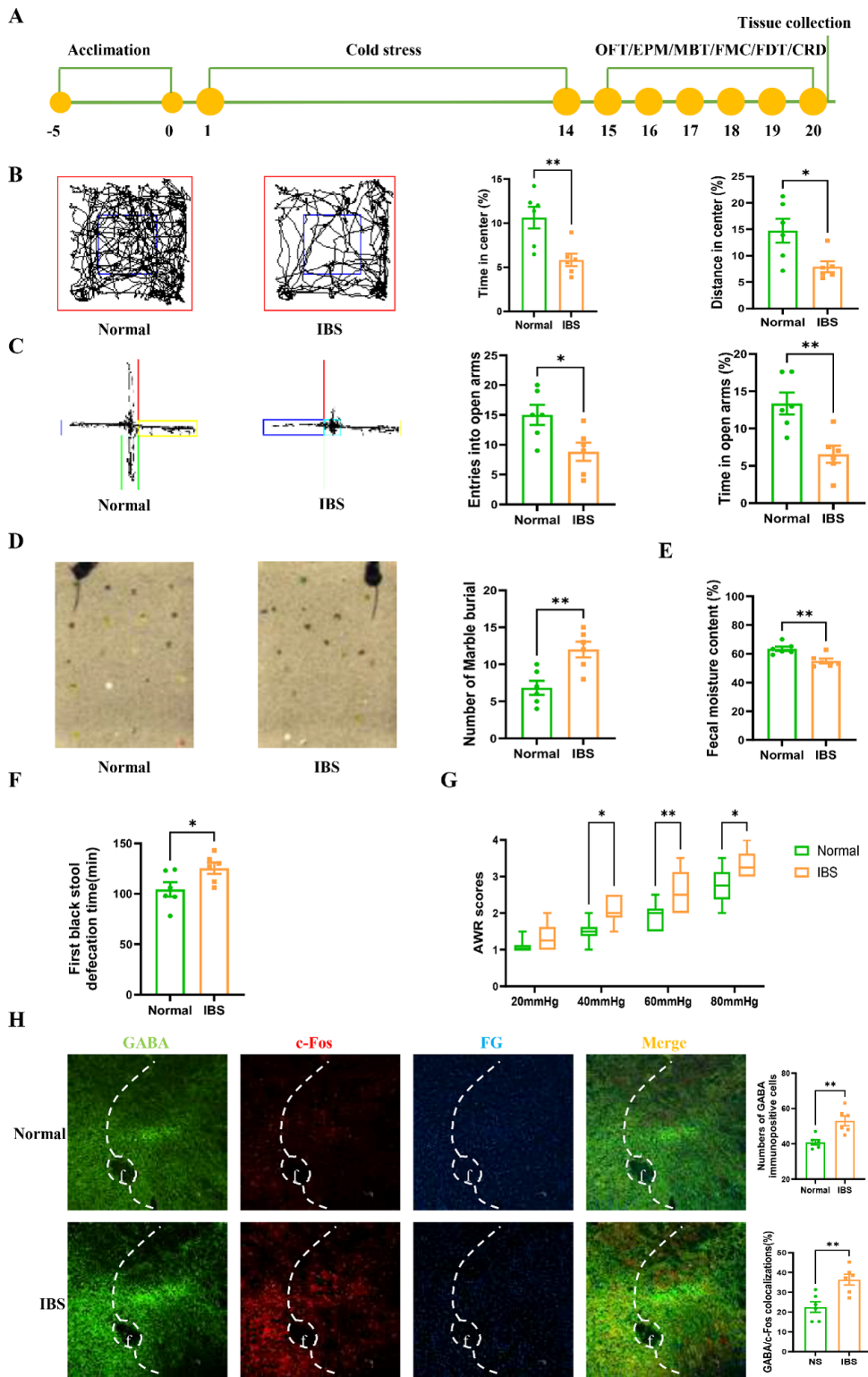


Figure 1

Cold water gavage stress induces anxiety-like behavior, constipation-like symptoms, and visceral hypersensitivity and neuronal activation of LHA GABAergic neurons

(A) Experimental timeline. (B) Time spent and distance traveled in the center zone of the open field. (C) Number of open-arm entries and time spent in the open arms of the elevated plus maze (EPM). (D) Number of marbles buried. (E) Fecal moisture content (FMC). (F) First black stool defecation time (FDT).

(G) AWR scores at graded colorectal distension pressures. (H) Left: representative immunofluorescence images of GAD67 (green) and c-Fos (red) in the LHA; right: quantification of GABA⁺ neurons per mm² and percentage of c-Fos/GAD67 co-labeled cells. Data are mean $\pm$ SEM (B–F, H) or median with 25th–75th and 5th–95th percentiles (G). n = 6 per group. * P < 0.05, ** P < 0.01 vs. Normal group.

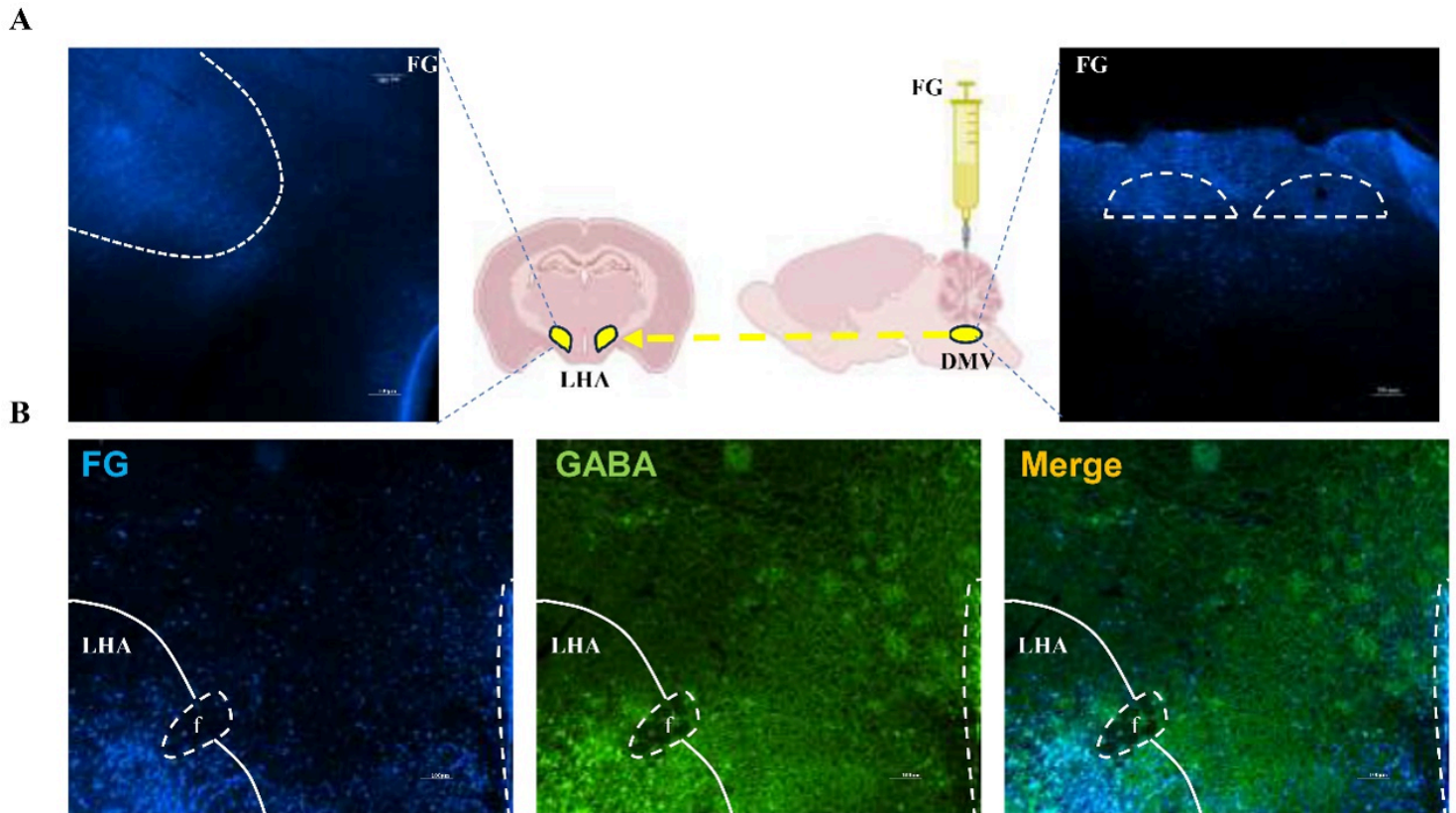


Figure 2

LHA GABAergic neurons project to the DMV

(A) Schematic and a representative image of the FG injection site in the DMV. (B) Left: representative images of FG (blue) and GAD67 (green) in the LHA; right: percentage of FG-labeled neurons among GAD67⁺ cells and percentage of GAD67⁺ neurons among FG-labeled cells. Data are mean $\pm$ SEM. n = 6.

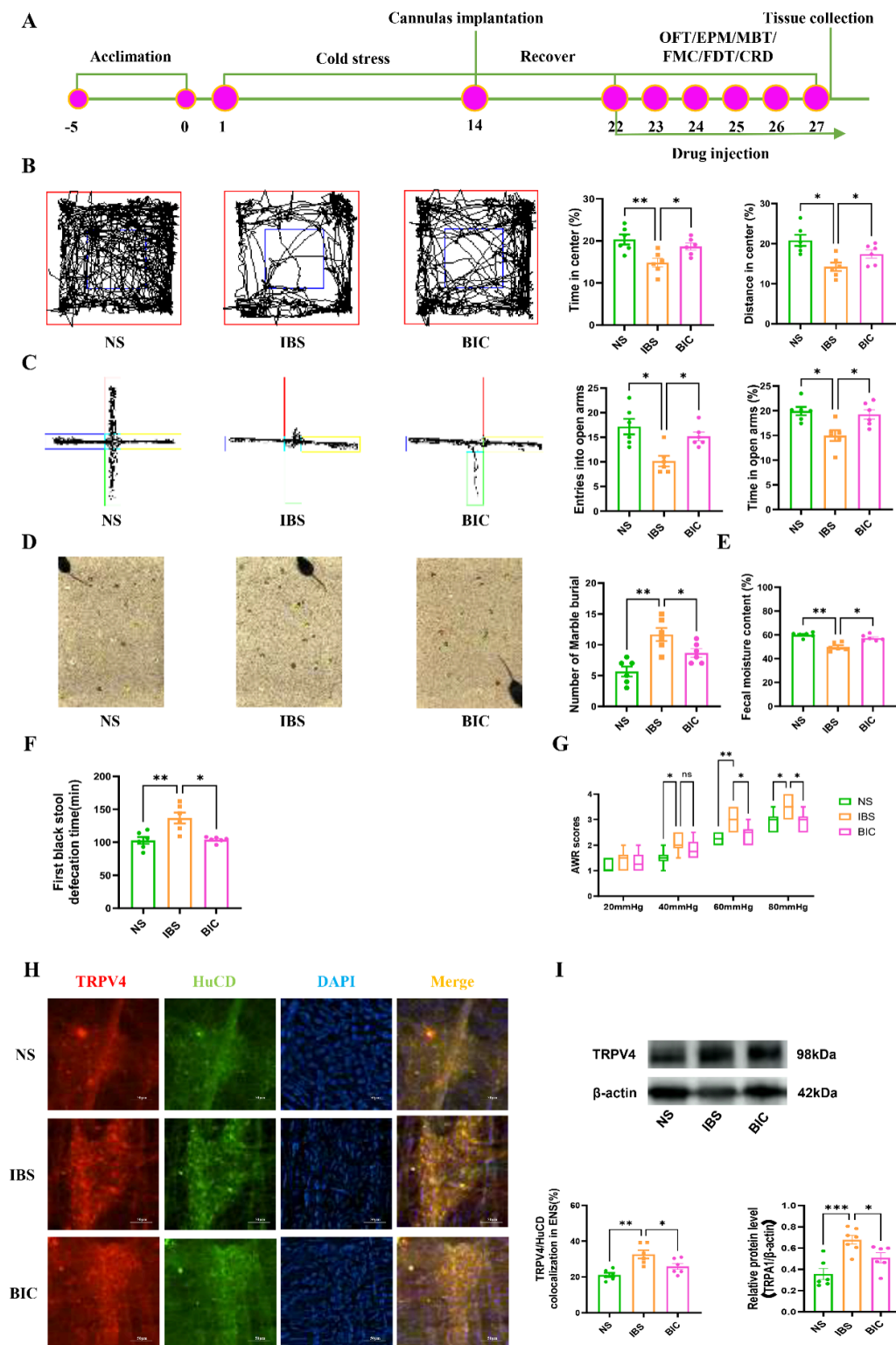


Figure 3

Pharmacological blockade of DMV GABAA receptors alleviates IBS-like symptoms

(A) Schematic of the experimental procedure for intra-DMV bicuculline (BIC) intervention in IBS-C model mice. (B) Center time and distance in the open field. (C) Open-arm entries and time in the EPM. (D) Number of marbles buried. (E) FMC. (F) FDT. (G) AWR scores. (H) Left: representative co-immunofluorescence images of TRPV4 and HuC/D in the enteric nervous system; right: percentage of

TRPV4/HuC/D co-labeled area. (I) Representative Western blots and quantification of colonic TRPV4 protein. Data are mean $\pm$ SEM (B–F, H, I) or median with percentiles (G). $n = 6$ per group. $*P < 0.05$ vs. IBS group.

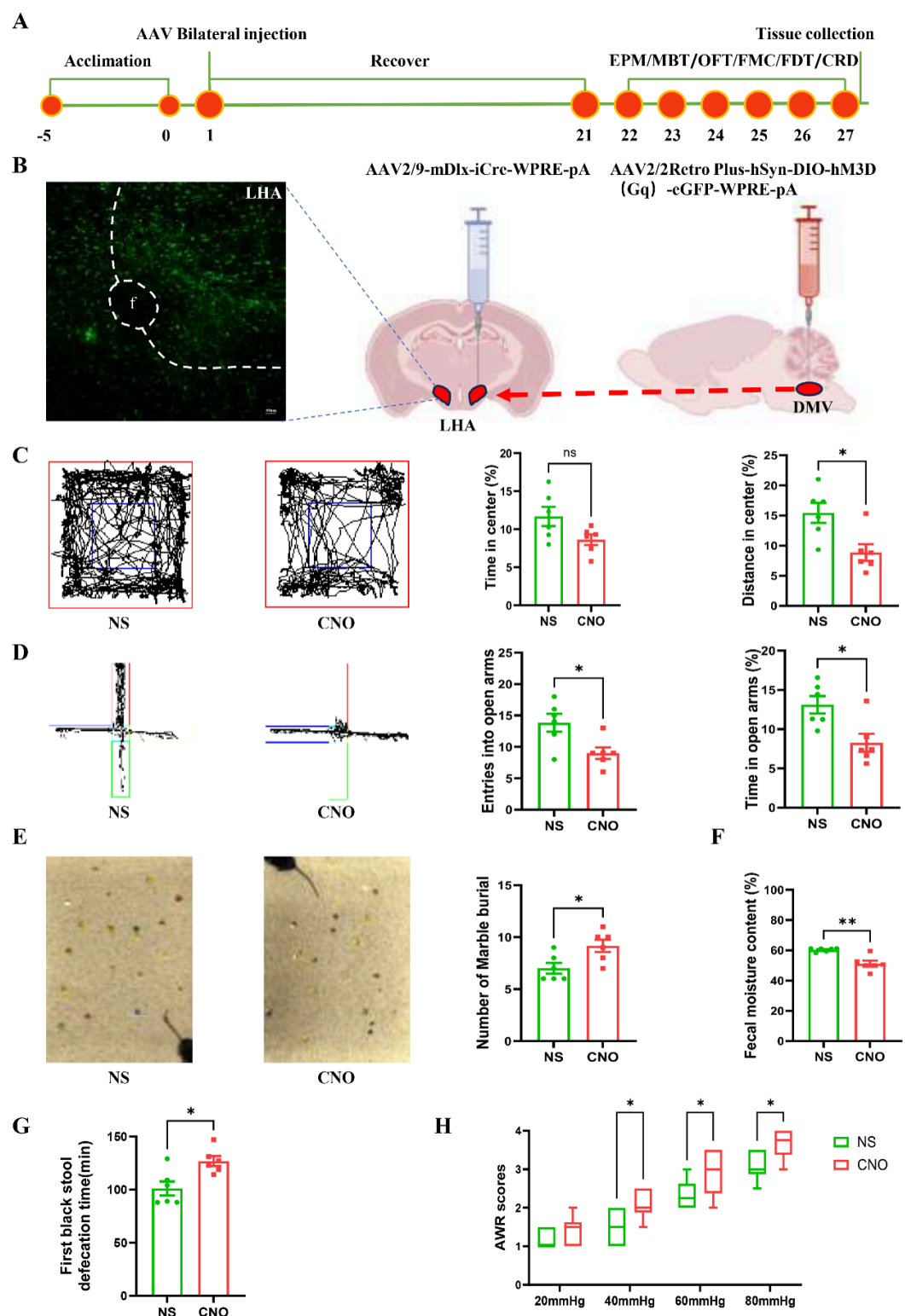


Figure 4

Chemogenetic activation of the LHA-DMV GABAergic pathway induces IBS-like symptoms in normal mice

(A) Experimental protocol. (B) Schematic of viral injection and a representative image showing eGFP expression in the LHA and DMV. (C) Center time and distance in the open field. (D) Open-arm entries and time in the EPM. (E) Number of marbles buried. (F) FMC. (G) FDT. (H) AWR scores. Data are mean $\pm$ SEM (C–G) or median with percentiles (H). $n = 6$ per group. $*P < 0.05$ vs. NS group.

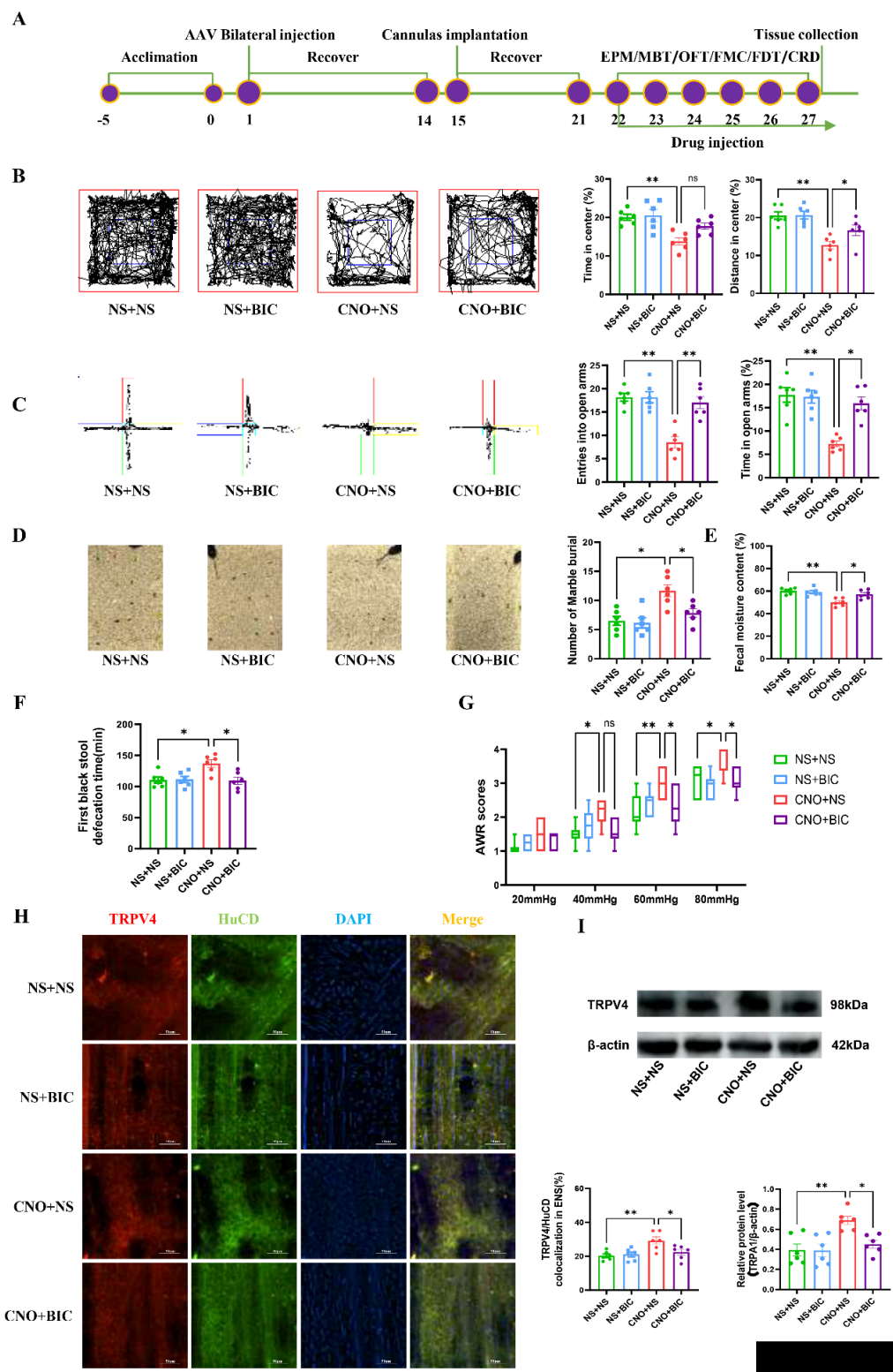


Figure 5

Blockade of DMV GABAA receptors reverses IBS-like symptoms induced by LHA–DMV GABAergic hyperactivation

(A) Experimental design for pharmacological rescue after chemogenetic activation. (B) Center time and distance in the open field. (C) Open-arm entries and time in the EPM. (D) Number of marbles buried. (E) FMC. (F) FDT. (G) AWR scores. (H) Left: representative TRPV4/HuC/D co-staining; right: percentage of co-labeled area. (I) Representative Western blots and quantification of colonic TRPV4 protein. Data are mean $\pm$ SEM (B–F, H, I) or median with percentiles (G). $n = 6$ per group. $*P < 0.05$, $**P < 0.01$ vs. CNO+NS group.