Effects of non-invasive vagal nerve stimulation on clinical, peripheral biologic, and brain measures of stress reactivity in irritable bowel syndrome: a randomized controlled trial

NCT #: NOT YET ASSIGNED

8/28/2026

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INSTRUCTIONS

- **This format may be used for**
  - **Non-exempt human subjects research (expedited or full board review)**
  - **Exempt human subjects research**
- ***Answer all questions***. If a question is not applicable to the research, state "N/A". If you do not answer a question, the IRB does not know whether the question was overlooked or whether it is not applicable. This may result in unnecessary "back and forth" for clarification. *Use non-technical language as much as possible*.
- For ***multi-site research***, describe only the activities under the UCLA investigator's purview unless you are requesting that a UCLA IRB provide the review and oversight for non-UCLA collaborators as well.
- **Please keep your responses succinct.**
- **You may use the index to jump to specific sections of the protocol**

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1. OVERVIEW

Title of Study

Effects of non-invasive vagal nerve stimulation on clinical, peripheral biologic, and brain measures of stress reactivity in irritable bowel syndrome: a randomized controlled trial

Lay language summary ☐ This is included in a separate scientific protocol uploaded to the IRB application.

Irritable bowel syndrome (IBS) is a chronic disorder of gut-brain interaction that causes abdominal pain and altered bowel habits. Many people with IBS also experience symptoms outside the bowel, such as headaches, widespread pain, poor sleep, anxiety, and depression; these symptoms are especially common in females. This study focuses on stress reactivity (SR), a persistent pattern of stress-system activity that may be linked to autonomic nervous system function, immune activity, the gut microbiome, and brain processing. We will enroll adults with at least moderate IBS symptoms and randomly assign them to 8 weeks of non-invasive vagal nerve stimulation (nVNS) or Usual Care. Participants assigned to nVNS will use a handheld device on the left side of the neck at home twice daily. We will compare changes in IBS severity and other physical symptoms and examine whether baseline or treatment-related changes in blood, stool, heart-rate variability, sex hormones, and brain imaging measures are associated with response. The study will also explore whether treatment effects and predictors differ by sex.

Study design. Provide a one-sentence description of the general study design and/or type of methodology.

This is a prospective, parallel-group, randomized controlled trial in 200 adults with IBS (100 females and 100 males) comparing 8 weeks of home-administered nVNS with Usual Care, with assessments at baseline, Week 4, Week 8, and Week 12 and brain MRI, blood, stool, and autonomic measurements at baseline and Week 8.

Specific Aims. Indicate the purpose of the research, specifying the problems and/or hypotheses to be addressed.

Project 1, Aim 3: Compare nVNS with Usual Care for reducing IBS and comorbid physical symptom severity and determine whether clinical and peripheral biologic measures of SR predict or change with treatment response.

• Hypothesis 1: Eight weeks of nVNS will produce greater reductions in IBS Severity Scoring System (IBS-SSS) and Patient Health Questionnaire-15 (PHQ-15) scores than Usual Care.

• Hypothesis 2: Baseline SR and peripheral biologic measures, including heart-rate variability, blood-based SNS-immune gene expression, and microbial and metabolite stress indices, will influence treatment response.

Project 1, Aim 3 endpoints:
Primary outcomes: IBS-SSS and PHQ-15 (see below)

1. Change in IBS-SSS: The IBS-SSS questionnaire is a validated measure of IBS symptom severity and has been shown to be responsive to treatment. The IBS-SSS assesses the severity of abdominal pain, frequency of abdominal pain, severity of abdominal distention, dissatisfaction with bowel habits, and interference of IBS with daily life over a 10-day period. Each of the five categories will receive a score from 0–100, and the total IBSSSS is calculated by taking the sum of these categories (range 0–500).
2. Change in Patient Health Questionnaire-15 (PHQ-15): The PHQ-15 is a widely used measure of widespread somatic symptoms over 1 month that is sensitive to treatment effects. PHQ-15 (and PHQ-12 without the three GI symptoms) have been used in IBS studies. Patients will complete the IBS-SSS and PHQ-15 at baseline, after 4 weeks of treatment, and after 8 weeks of treatment.

Secondary endpoints:

1. IBS-SSS responder rate (proportion of patients with IBS who experience ≥50-point reduction in IBS-SSS), which represents a clinically meaningful improvement in IBS symptoms
2. Biologic measures of SR: SNS–Immune gene expression profile, HRV, fecal microbial stress index, and fecal metabolite stress index.

Project 2, Aim 3: Determine the effects of nVNS on SR-related brain signatures and evaluate IBS- and sex-related differences in these effects.

• Hypothesis 1: Compared with Usual Care, nVNS will produce larger changes in SR-related brain activity and connectivity.

• Hypothesis 2: Brain changes will correlate with improvements in IBS severity, comorbid physical symptoms, and peripheral biologic measures of SR.

• Hypothesis 3: Baseline SR-related brain signatures will predict response to nVNS.

• Exploratory hypothesis: Females with IBS and greater comorbid symptom burden will show greater treatment-related changes in SR-related brain signatures.

Project 2, Aim 3 endpoints:

Primary endpoints:

1. Compare the difference in pre–post-treatment change in SR-related brain phenotypes (structural, connectivity, activity) between the nVNS and Usual Care treatment arms.

Secondary endpoints:

1. Examine associations between changes in scores on the SR-related brain phenotype signatures and changes in IBS severity, comorbid symptom scores, and peripheral biologic measures of SR.
2. Predict response (Pre-Post change in extraintestinal symptoms) in nVNS compared to Usual Care.

Exploratory endpoint: Examine whether females with IBS with higher comorbid symptom scores will present with greater changes in SR-related brain signatures following the nVNS intervention compared to male IBS.

Background Provide the rationale and the scientific or scholarly background for the proposed activity, based on existing literature (or clinical knowledge). Describe the gaps in current knowledge that the project is intended to address. *This should be a plain language description. Do not provide scholarly citations. Limit your answer to less than one page or refer to an attached document with background information that is no more than three pages long.*

☐ This is included in a separate scientific protocol uploaded to the IRB application.

Current IBS treatments primarily target bowel symptoms and are effective for only a subset of patients. Many patients also have extraintestinal physical and psychological symptoms that are not adequately addressed. Heightened SR may contribute to this broader symptom burden through persistent sympathetic nervous system activity, altered immune signaling, changes in the gut microbiome and metabolites, and altered processing within brain networks that regulate pain, emotion, and autonomic function.

The vagus nerve is a major pathway linking the brain, autonomic nervous system, immune system, and gastrointestinal tract. nVNS is a non-invasive neuromodulation approach that has shown benefit in headache, chronic pain, mood disorders, and preliminary studies of IBS and gastroparesis-like symptoms. Pilot work by our research

group found that a single 4-minute nVNS administration was well tolerated by females with IBS and was associated with reduced abdominal pain and measurable changes in insular, prefrontal, and brainstem activity and connectivity.

It will test whether 8 weeks of nVNS improves IBS and comorbid physical symptoms compared with Usual Care and whether treatment response is associated with peripheral biologic measures and SR-related brain signatures. It will also evaluate sex differences in response and potential predictors of benefit. These findings may support future development of biologically informed, personalized treatments for IBS with comorbid symptoms.

Investigators' Experience and preliminary work Briefly describe experience or preliminary work or data (if any) that you, your team, or your collaborators/co-investigators have that supports the feasibility and/or safety of this study.
☐ This is included in a separate scientific protocol uploaded to the IRB application.

Higher comorbid symptom burden in females with IBS than in males with IBS: The location and severity of common comorbid symptoms in IBS vary across individuals. Although the number of concurrent diagnoses is sometimes used to assess comorbidity, a symptom-based approach is more sensitive and comprehensive. We used a validated, broad-based symptom assessment, the Complex Medical Symptom Inventory (CMSI), which measures the presence of somatic, visceral, neuropathic, and sensory symptoms within the last 3–12 months, as our primary measure of comorbid symptom burden. In a cohort of 365 individuals with IBS (71% females) and 341 healthy controls (HCs) (58% females), we found higher total CMSI scores in individuals with IBS than in HCs ($p<0.001$) and in females with IBS than in males with IBS ($p<0.001$) but no difference between male and female HCs.

SR is a significant moderator of sex difference in comorbid symptoms in IBS: Using principal component analysis of Perceived Stress Scale (PSS) and International Personality Item Pool (IPIP-N) scores, we defined high- and low-SR groups. Individuals with IBS in the high-SR group had worse IBS severity (measured by the IBS Severity Scoring System [IBS-SSS]; $p=0.04$), poorer health-related quality of life (HRQOL; measured by the IBS quality of life questionnaire [IBS-QOL]; $p=0.04$), and higher comorbid symptom burden (CMSI score) than the low-SR group. This SR phenotype moderated sex differences in comorbid symptom burden (CMSI score, Patient Health Questionnaire [PHQ-12] score), as females had higher CMSI scores than males in the high-SR group but not the low-SR group.

SR-related differences in global connectivity of CAN and brainstem regions: Compared with the low-SR group, the high-SR group had reduced global connectivity of the posterior insula (pINS) and anterior insula (aINS) subregions and regions within the CAN ($q<0.05$). We also observed moderate effect size reductions (Cohen's $d=0.32–0.43$) in the high-SR group that were significant at nominal p-values ($p<0.05$) in other emotional arousal regions (right hippocampus, right amygdala, bilateral parahippocampal gyrus, left rostral anterior cingulate cortex (ACC)), subregions within the pINS and aINS, regions in the default mode network (bilateral precuneus, left medial prefrontal cortex (mPFC)), and brainstem nuclei (right parabrachial nucleus (PBN), and median raphe nuclei (MRN)). In a subset of individuals with blood transcriptomics data and neuroimaging data, including 34 individuals in the low-SR group (23 females, 17 with IBS) and 16 individuals in the high-SR group (12 females, 14 with IBS), reductions in global connectivity of the pINS, aINS, and right PBN were significantly associated with increased whole-blood SNS–immune gene expression at $q<0.05$ (standardized beta[stdB]:−0.51, −0.37). We also demonstrated that the high-SR group had alterations in the CAN that demonstrated an increase in neuronal or axonal density in sensory processing areas and brainstem ascending arousal systems.

- Direct comparison of the whole-brain connectivity of aINS subregions revealed sex differences in left dorsal anterior insula (daINS) and left ventral anterior insula (vaINS) connectivity with the superior parietal cortex (central executive/attentional network) and prefrontal regions ((ventrolateral prefrontal cortex (vlPFC) and ventromedial PFC (vmPFC)). Sex differences were also observed in INS subregions. Intrinsic connectivity

analyses of INS regions of interest (ROIs) revealed that females with IBS had reduced connectivity of the left and right vaINS to bilateral pINS compared with males with IBS, but no sex-related differences in the connectivity of the left and right daINS to bilateral pINS were identified, suggesting that although sex does not impact connectivity of the primary interoceptive cortex (pINS) with the dorsal aINS (which is linked to cognitive brain networks), females show reduced connectivity of the pINS with the ventral aINS (which is linked to emotional-regulation networks).

SR-related brain phenotype is associated with gut microbial stress index: Using our previously published approach, we defined a microbial stress index for estimating the probability of high SR using a random forest classifier based on bacterial species that were enriched or depleted in individuals with high SR. The greatest contributor to the classifier was *Roseburia hominis*, a butyrate producer depleted in individuals with high SR. Moderator analyses indicated that a high microbial stress index was associated with increased CMSI and PHQ-12 (PHQ-15 without the 3 GI symptoms) scores in females but not in males ($p<0.01$). The microbial stress index was not associated with increased IBS symptom severity (IBS-SSS) in either males or females, indicating that its relationship with IBS symptoms is not driven by increased IBS severity. Scores of this brain signature showed a small effect size correlation ($r=0.24$ 95% confidence interval [CI], 0.10–0.37, $p=0.001$) for the relationship with the microbial stress index. The signature comprises the pINS, aINS, amygdala, hippocampus, parahippocampus, ventral tegmental area (VTA), and PBN. These results suggest that altered BGM interactions reflect either top-down modulation of the microbial ecosystem by the brain or bottom-up signaling from SR-associated microbiota and their metabolites to the brain, or a combination.

VNS usage with IBS patients: A percutaneous taVNS device, IB-Stim (NeurAxis Inc, Carmel, IN, USA) is FDA-approved for the treatment of functional abdominal pain associated with IBS in adolescents, reducing abdominal pain scores after 3 weeks of treatment. A 4-week taVNS RCT in adults with IBS in China found that bilateral taVNS treatment (n=21) significantly improved constipation, abdominal pain, anxiety, and depression compared to baseline and sham taVNS (n=21). taVNS significantly increased vagal tone (high frequency (HF) of HRV) compared to baseline and sham taVNS, and post-treatment vagal tone modestly correlated with improved abdominal pain and stool frequency. We conducted a pilot RCT using the IB-Stim device in adults with IBS. Our pilot data in 12 participants with IBS (6 taVNS, 6 Sham devices) showed a higher responder rate (IBS-SSS score; primary endpoint) in the taVNS group (60%) vs the sham group (40%) (estimate=−46.79, $p=0.23$). IBS-QOL improved by week 4 (Estimate=11.75, $p=0.23$) and week 8 (Estimate=21.31, $p=0.07$), suggesting that VNS is a promising therapy in adults with IBS.

nVNS efficacy: The GammaCore (electroCore LLC, Basking Ridge, NJ, USA) device, which can be used 2–3 times daily and does not require in-person clinic visits, is FDA-approved for the treatment of multiple types of headaches, including migraines. Three open-label, small pilot studies are using this nVNS device in patients with gastroparesis symptoms, showing reductions in gastroparesis-like symptoms, depression scores, and migraines, with no differences between right- and left-sided stimulations.

Our nVNS pilot study: We piloted a functional magnetic resonance imaging (fMRI) study in 6 premenopausal females with IBS, performing resting state fMRI before a standard 4-minute nVNS stimulation using Gammacore on the left side of the neck, followed by repeated fMRI during an emotion–response task. On average, participants reported a 12.5% reduction in abdominal pain intensity relative to baseline after one-time nVNS. All participants reported nVNS as tolerable, without side effects, and indicated they would definitely consider participating in an 8-week trial.

- **Effect of nVNS on INS, key CAN regions, and brainstem resting-state connectivity:**
    - After nVNS, the left vaINS had reduced connectivity with the right medial PFC ($q<0.0001$) and increased connectivity with the left dorsal mPFC ($q<0.0001$). The right vaINS had increased connectivity with the central executive/dorsal attention network (bilateral superior parietal cortex,

q<0.0001) and sensorimotor left precentral gyrus (q<0.0001). No changes were seen for pINS (poster INS) and mINS (mid INS) connectivity after nVNS.

- During baseline (before nVNS), more activation in CAN regions was observed when viewing negatively valenced images than during or when viewing neutral or positively valenced images (Z>2.3, p<0.05, Cohen's d=0.94). Activated regions included the bilateral brainstem and CAN regions, such as the amygdala, INS subregions, medial prefrontal cortex, and perigenual ACC.
  After nVNS application, reduced activity was observed in these regions (Z>2.3, p<0.05), associated with altered connectivity between the INS subregions and brainstem nuclei

In the current study proposal, we will be using Truvaga Plus nVNS device, which is also made by electroCore, LLC, and exerts similar nVNS effects but is rechargeable. This allows us to reuse this device among participants.

Specific Categories of research. Check all boxes that apply. For those that require completion of a supplement, the content of those supplements should be uploaded to ***BruinIRB***.

Check all that apply	Type of Research	Supplement Name and Link
☐	Emergency exception to informed consent	N/A – no supplement
☐	Deception or Incomplete Disclosure	SUPPLEMENT Consent Waivers
☐	Waivers of Consent/Waivers of documentation of consent	SUPPLEMENT Consent Waivers
☒	**None of the above**	

2. PARTICIPANTS

Describe the **general characteristics** of the subject populations or groups, including age range, gender, health status, geographic location, and any other relevant characteristics.
☐ This is included in a separate scientific protocol uploaded to the IRB application.

Adults aged 18-50 years with IBS will be recruited. The study will enroll 100 premenopausal females and 100 males, with equal allocation by sex to nVNS and Usual Care. Participants will be recruited primarily from the greater Los Angeles area and must be able to complete study procedures at UCLA.

Inclusion criteria. Describe the specific criteria that will be used to decide who will be included in the research from among interested or potential subjects. Define any technical terms in lay language.
☐ This is included in a separate scientific protocol uploaded to the IRB application.

- Age 18-50 years.
- Meets Rome V diagnostic criteria for IBS-D or IBS-M.
- At least moderate IBS symptom severity, defined as IBS-SSS ≥175.
- For females: premenopausal with generally regular menstrual cycles and willing to complete study visits within the study-specified menstrual-cycle window when feasible.
- Able to understand and provide informed consent in English.
- Willing and able to complete questionnaires, biospecimen collection, wearable monitoring, MRI procedures, and randomized study requirements.

Exclusion criteria. Describe the specific criteria that will be used to decide which of the subjects who meet the inclusion criteria listed above will be excluded from the research. Define any technical terms in lay language. There is no need to list the opposite of the inclusion criteria.

☐ This is included in a separate scientific protocol uploaded to the IRB application.

- Individuals with obesity (BMI > 30).
- Evidence or history of another gastrointestinal disease that could explain symptoms, including inflammatory bowel disease or celiac disease, unless adequately excluded by clinical evaluation or prior testing.
- Pregnancy or a positive pregnancy test.
- Perimenopausal or postmenopausal status.
- Previous use in the last 3 months OR current use of hormonal medications, including hormonal contraceptives (i.e. birth control pills, patches, implants, or intrauterine devices (IUDs)) or hormone replacement therapy
- MRI contraindication, including a non-removable ferromagnetic implant or device, prior significant tattoo-related heating or burns, severe claustrophobia that cannot be managed, or another condition judged unsafe for MRI.
- Major active psychiatric illness (e.g., schizophrenia, bipolar disorder), significant neurologic or central nervous system disease, or another medical condition that could affect safety, adherence, or interpretation of study outcomes.
- Current participation in another IBS-specific intervention study or planned initiation of a new IBS-directed treatment during the 8-week randomized period.
- Contraindication to cervical nVNS or inability to use the device safely.
- Dermatologic conditions affecting the neck region that would interfere with nVNS placement
- Any implanted electrical device
- Any metallic device implanted at or near the neck
- History of cervical vagotomy
- Use of another electrical device at the same time (e.g., TENS unit, muscle stimulator) or any portable electronic device
- Movement disorder
- Clinically significant hypertension, hypotension, bradycardia, or tachycardia.
- Cardiac disease or atherosclerotic cardiac disease, including congestive heart failure, known severe coronary artery disease, or recent myocardial infarction within 5 years.
- Past abdominal surgery (except for appendectomy, cholecystectomy, benign polypectomy, hiatus hernia)
- Inability or unwillingness to provide informed consent or complete study procedures in English.

Number of subjects. For each subject group, use the table below to provide the estimate of the maximum desired number of individuals (or other subject unit, such as families) who will complete the research. [*Click on the "+" on the left end of the dotted line to add more rows (hover cursor over that area for the "+" to show)*]

Trial Arm	Group name/description	Maximum desired number or individuals (or other subject unit) who will complete the research *Provide numbers for the site(s) reviewed by a UCLA IRB and for the study-wide total number; example: 20/100*
Usual Care	IBS female	50
	IBS male	50
nVNS	IBS female	50
	IBS male	50

Special Participant Populations. Check all boxes that apply and complete and upload any required SUPPLEMENTS

Check all that apply	Type of Population	Supplement Name and Link
☐	**Pregnant individuals, fetuses, fetal tissue, and/or Neonates**	SUPPLEMENT Pregnancy and Neonates
☐	**Prisoners**	SUPPLEMENT Prisoners
☐	**Children (minors)**	SUPPLEMENT Children/minors
☐	**Adults not able to consent for themselves**	SUPPLEMENT Adults not able to consent
☒	**UCLA employees or students**	N/A – no supplement
☒	**Native American and/or non-U.S. Indigenous Populations**	N/A – no supplement
☐	**None of the above**	

3. PROCEDURES

Study procedures. Using lay language, provide a complete description of the study procedures, including plans for randomization, the sequence, intervention or manipulation (if any), drug dosing information (if any), blood volumes and frequency of draws (if any), use of records, time required, survey instruments/questionnaires, assessment of efficacy/safety, and setting/location. If it is available: Upload a study flow sheet or table of procedures to **BruinIRB** or paste it in the box below.

☐ This is included in a separate scientific protocol uploaded to the IRB application

Total study participation is expected to take 12-20 weeks.

a) Participants for the study will either come from IRB #26-1173 or will be recruited directly to participate in this study. For participants who are recruited specifically for this study, they will undergo a screening visit as outlined in IRB #26-1173 (see procedures below).We will recruit equal numbers of males and females with IBS from the community rather than relying on clinic referrals to minimize bias and allow for a more accurate determination of whether females truly experience a broader range of comorbid symptoms (ranging from no to multiple symptoms) than males. We will also include overall healthcare-seeking behavior as a covariate, assess differences between males and females, and examine its impact on comorbid symptoms and disease severity.

**Female participants will be required to come in for their visit during their luteal phase. Female participants will be required to download the free application, Clue Period Tracker & Calendar, on their personal smartphone device. They will then fill out their personal menstrual history, including day one of their menstrual period, period length, and cycle length. Additionally, participants will be asked to add the first and last date of their last 3 menstrual periods.*

Before study participation begins: Phone Screening
All interested participants will complete a call over the phone with a study team member (CRC, research assistant) to assess their baseline eligibility for the study (see ”phone screen” document under IRB recruitment materials). Those who complete the phone screening will not be consented prior to the call; however, if someone is determined to be eligible based on the phone screening and is interested in moving forward to participate, they will then proceed into the study and be consented via study procedures outlined below (see ”Consent and questionnaire completion”). All people who complete the phone screening will be informed of their eligibility status after the call (eligible vs ineligible), but will not be informed as to why the decision was made. This will ensure that specific eligibility and ineligibility criteria doesn’t become public knowledge and cause interested people to withhold pertinent information from the study team.

Consent and questionnaire completion:

1. After a participant has completed the phone screening, been identified as eligible, and has indicated they would like to move forward with the study procedures, their consent will be obtained via the platform, Docusign.
    a. All participants will receive a copy of the consent form and study description.
2. For participants completing informed consent remotely, authorized study personnel will send the informed consent form electronically through DocuSign and schedule a telephone call to conduct the consent discussion. The DocuSign message will instruct participants to review the consent form but not sign it until they have discussed the study with the study team and all questions have been answered.
3. During the telephone consent discussion, the participant will have access to the electronic consent form while authorized study personnel review the study purpose, procedures, duration, risks, potential benefits, alternatives, confidentiality protections, voluntary nature of participation, and right to withdraw. Participants will be encouraged to ask questions throughout the discussion. Study personnel will assess the participant’s understanding by asking the participant to explain, in their own words, the study’s main procedures, risks, and voluntary nature. Study personnel will clarify any misunderstandings before obtaining consent.

4. Only after the consent discussion is completed, all questions have been answered, and the study team confirms that the participant appears to understand the study will the participant electronically sign and date the consent form. Authorized study personnel will then countersign the form, and the participant will receive a completed copy through DocuSign. The date and method of the consent discussion and the names of those participating will be documented in the study record. No screening or other study procedures will begin until informed consent has been completed.
5. Consent will be obtained after participants complete the phone screening but before the online Zoom screening. Prior to the Zoom screening, participants will complete questionnaires that will be used to help determine eligibility (e.g., BSQ). Participants must provide informed consent before any study questionnaires are completed or other study procedures are completed. Thus, the order of procedures will follow: phone screening → consent discussion and electronic consent → completion of questionnaires → Zoom screening visit.'
6. Research Coordinators will complete the consenting process. Research Coordinators involved in the consent process have completed all required institutional training for human subjects research, including Good Clinical Practice (GCP), Human Subjects Protection, HIPAA/privacy training, and study-specific protocol training. Research Coordinators are trained in informed consent procedures and are knowledgeable about the study protocol, procedures, risks, benefits, eligibility criteria, and participant rights. They are qualified to explain study-related information, answer questions from potential participants, assess participant understanding, and refer clinical or medical questions to the Principal Investigator or other qualified study clinician when appropriate. All consent activities are conducted under the supervision of the Principal Investigator and in accordance with institutional and IRB requirements.
7. Participants who were enrolled from IRB #26-1173 will be consented in-person during their final visit for IRB 26-1173. They will review a copy of the consent form sent via DocuSign and will review the consent with the study team prior to signing. The study team will ask the participant questions on the study scope to ensure understanding prior to signing. All other participants will have signed consent during the Zoom screening as indicated above. For participants who are completing consent remotely, the consent process will be conducted via telephone and DocuSign as described above.
8. All participants will complete the following questionnaires sent to their email via REDcap. Questionnaire will be sent to participants about 1 week prior to their Zoom visit:
    a. UCLA Bowel Symptom Questionnaire (BSQ)
    b. Adverse childhood experiences (ACE)
    c. International Personality Item Pool-Neuroticism (IPIP-N)
    d. Perceived stress scale (PSS)
    e. Connor-Davidson Resilience Scale (CD-RISC)
    f. Brief Resilience Scale (BRS)
    g. Complex Medical Symptom Inventory (CMSI)
    h. Comorbidities of Pain and Chronic Conditions (COPC) Screener
    i. Personal Health Questionnaire (PHQ-15)
    j. Generic quality of life (The SF-12 (Short Form-12))
    k. Hospital Anxiety and Depression Scale (HADS)
    l. The Vioscreen Dietary Questionnaire
    m. UCLA Diet Checklist
    n. Current medication history
9. IBS participants will also complete the following questionnaires:
    a. IBS Severity Scoring System (IBS-SSS)

b. Irritable Bowel Syndrome Quality of Life (IBS-QOL)
c. Bristol Stool Form Scale in the past 24 hrs
d. Overall GI symptom severity in the past 24 hrs (0-10 numeric rating scale)
e. Overall abdominal pain severity in the past 24 hrs (0-10 numeric rating scale)

Note: All study questionnaires are scored while the subjects are still participating in the study and attending study visits, as such, follow-up/referrals will be provided for subjects whose responses indicate moderate or higher levels of anxiety or depression

Zoom Screening Visit (Virtual): Home; 1-1.5 hours
*NOTE: ONLY for participant who did not complete IRB 26-1173

10. This Zoom screening visit will occur after consent and questionnaire completion. Although this Zoom visit is not the primary consent discussion, participants will again be asked if they have any additional questions during the visit or at any time during study participation.
    a. Participants will then undergo a screening visit (via Zoom) that entails a review of the participant's medical history. Additionally, all participants will undergo a brief structured psychiatric exam (Mini-International Neuropsychiatric Interview [M.I.N.I.]). The MINI+ is a validated, structured screening/assessment instrument based on DSM criteria. If a psychiatric condition is present, it may result in a positive endorsement on the structured interview. The MINI+ will be used as a standardized screening/assessment instrument for study purposes and will not be used by Research Coordinators to make clinical diagnoses. All MINI+ results will be reviewed by the Principal Investigator, Dr. Chang, who is responsible for the clinical interpretation of the findings and any appropriate follow-up actions.Lin Chang MD and Arpana Church, PhD, will remotely administer the MINI+ during the Zoom screening. The MINI will be reviewed and scored during a participant's study visit. The MINI+ is a validated tool based on DSM. If an illness is present, it will show as a positive endorsement on the structured interview. The MINI+ will be administered by a qualified clinician PhD, Dr. Arpana Church or Dr. Lin Chang (PI). Both Dr. Church and Dr. Chang have been trained in its administration by their academic preparation. The MINI+ is used as a standardized screening/assessment instrument for study purposes and is not used by the coordinators to make clinical diagnoses. All results are reviewed by the PI, who is responsible for the clinical interpretation of the findings and any follow-up actions, as appropriate. If responses indicate current suicidality during the remote visit, the study team will provide appropriate resources. In cases of emergencies, 911 would be contacted immediately. Other resources that will be provided will be the phone numbers to the National Suicide Prevention Lifeline (1-800-273-TALK), the Los Angeles County Suicide Prevention Hotline (877-7-CRISIS), or UCLA Counseling & Psychological Services (CAPS) (310-825-0768; UCLA students only).
    b. The study physician, Lin Chang MD, will complete the medical history aspects of the zoom screening whilst study clinician Arpana Church PhD, will only assist in the completion of the MINI+.
    c. Participants identified through the MINI as having major depression, significant depressive symptoms, alcohol use disorder, substance use disorder, or other clinically significant psychiatric findings that do not require emergency intervention will be informed of the findings and provided with appropriate referrals for further evaluation and treatment. Referrals may include the participant's primary care provider, treating mental health provider,

behavioral health services, substance use treatment programs, or other appropriate community or institutional resources. The referral will be documented in the study records.

IN-PERSON SCREENING VISIT: (UCLA Westwood; 30 minutes-1 hour)

1. **Screening Tests:**
   All female participants will complete a urine pregnancy test
   - If the test is positive, they will not be allowed to continue with the study
   - The test will be repeated at each subsequent study visit to ensure participant safety

SAMPLE TYPE	DESCRIPTION	PARTICIPANT GROUP
Blood	Complete Blood Count (CBC); 2-4mL	All groups (if not completed within the past year)
	Celiac Serology, C-reactive Protein Level; 3.5-4mL	IBS participants (if not completed in the past or results are not accessible)*
Stool	Fecal Calprotectin	IBS participants (if not completed in the past or results are not accessible)*

*If a participant has had significant changes to their symptoms (i.e., unexplained weight loss, bloody stool) since last getting tested, repeat labs will be performed. If blood/ stool samples show elevated inflammation or other signs of underlying illness, a patient may be removed from the study.

If it is determined that a participant needs to complete a stool sample a stool sample collection kits (1 Para-Pak and 1 Ethanol collection vial) will be provided to participants prior to their appointment (by mail or pick up) and participants will be instructed to collect the sample 24-48 hours before their visit. Kits will include coolers and ice packs to transport samples to in-person visits.

BASELINE VISIT (Visit 1): UCLA Westwood; 2.5-3 hours

2. If participants were specifically recruited for this study:
   a. A physical exam will be performed by a licensed healthcare provider if the participant has not had a physical exam by their primary care physician (PCP) within the past year.
   b. Body Mass Index (BMI) will be collected
3. **Blood draw:** Blood samples will be collected for whole-blood SNS–immune gene expression profiling and estrogen and androgen metabolites.
   a. The following tubes will be collected: PAXgene RNA, EDTA , Gold top (Serum Separator Tube (SST))
   b. In total, up to 20 mL of blood will be collected (~4 teaspoons)
4. **Stool samples:** Stool samples will be collected for shotgun metagenomics, metabolomics, and estrogen and androgen metabolites. Stool sample collection kits (1 Para-Pak and 1 Ethanol collection vial) will be provided to participants prior to their appointment (by mail or pick up) and participants will be instructed to collect the sample 24-48 hours before their visit. Kits will include coolers and ice packs to transport samples to in-person visits.

5. **Heart Rate Variability (HRV)**: Physiological data will be collected using commercially available Fitbit devices equipped with photoplethysmography (PPG) optical sensors, which detects peripheral blood volume changes to estimate heart rate and the intervals between successive beats.
    a. Participants will wear the Fitbit starting at Visit 1 (baseline) until Visit 2 (post-intervention).
    b. To collect sleep staging and longitudinal circadian HRV, participants will be instructed to wear the Fitbit continuously (including during sleep) and may only remove the device temporarily for charging or while showering.
6. **MRI Scan** (90 minutes)**:**
    a. Scanning sessions will acquire data throughout the brain and brainstem. All subjects will be imaged on a Siemens 3 Tesla Prisma scanner housed at the UCLA Ahmanson- Lovelace Brain Mapping Center (ALBMC). Participants will be asked to fast for 4-6 hours before the MRI.
        - All participants will be provided with earplugs and headphones to reduce noise from the MRI scanner, but they will still be able to hear and talk to the study team
        - An emergency squeeze ball will be placed within reach of the participant for stopping the scan quickly if they do not want to continue with the scan
    b. Participants will undergo a set of MRI scans, including a structural scan, two functional resting state scans, a functional scan during the emotion–response task (looking at positive, negative, and neutral pictures), and two 5-minute diffusion tensor imaging scans.
        - Functional scan: Participants will watch a total of six Pblocks of negative, neutral, and positive valence pictures selected from the International Affective Picture System (IASP). Each block contains four different pictures of the same valence displayed for 40 seconds each. The order of blocks is positive, neutral, negative, negative, neutral, and positive. There are three 15-second resting blocks separating the first three blocks, a 60 s resting block, and three 15 s resting blocks separating the last 3 blocks
7. **Randomization to study arms:** Participants will be randomly assigned (1:1) to nVNS or Usual Care arms using a computerized randomization protocol, in blocks of two and four.
    a. **nVNS arm:**
        - Device: Participants will self-administer a transcutaneous cervical nVNS device, Truvaga Plus, which is identical to GammaCore but is rechargeable. Truvaga Plus is classified as a general wellness product that does not require FDA clearance and can be purchased without a prescription (electroCore LLC, Basking Ridge, NJ, USA, letter of support).
        - Usage: The nVNS will be delivered via two stainless steel contact surfaces that are coated with conductive gel before each treatment. The device will be positioned in parallel with the carotid pulse on the left side of the neck. Two back-to-back 2-minute nVNS stimulations (4 minutes total) will be administered twice daily at home for 8 weeks.
            - Intensity: Level is dependent on the patient. They will be instructed to increase the intensity until they feel a strong but comfortable sensation—tingling or a slight lip pull is common. Most users find the ideal intensity between levels 4 and 7.
            - Frequency: 2 sessions, 2x daily; 224 sessions total over 8 weeks
            - Duration: 2 2-minute session, 2x daily; 4 minutes daily; 224 minutes over 8 weeks
        - Participant Training: All participants will be trained on proper positioning and will be instructed to adjust the stimulation intensity voltage to achieve a comfortable tingling

sensation in tissues beneath the stimulation plates and the engagement of the facial nerve (evidenced by ipsilateral lip pull). Our skilled study coordinator will provide training on the correct use of the nVNS device.

- Compliance will be assessed through an online survey completed daily and weekly. Participants who do not complete the survey on any given day will receive email, text, or telephone follow-ups from the study team. After the first 4 weeks, the participant will be allowed to increase the frequency of nVNS stimulations to three times daily, similar to other nVNS studies, if they do not experience adequate relief of IBS symptoms. Adherence to study guidelines will be tracked and recorded by the study team.
- Minimum participation requirements: Participants are required to use nVNS daily and complete daily symptom questionnaires at least 5 days per week and weekly questionnaires throughout the 8-week trial to be included in the per-protocol analysis. If this requirement is not met, that participants may be replaced to maintain the target sample size, while all randomized participants remain in the primary intention-to-treat analysis regardless of adherence or replacement status However, participants with incomplete data will still be included in the primary analysis.
    - Questionnaires: Participants will receive a daily questionnaire to their email which will assess daily symptoms and weekly symptom questionnaires. They are allowed to miss 2 daily questionnaires each week throughout the entire 8-week period.

b. **Usual Care arm:**

- Participants assigned to the Usual Care arm will undergo the same assessments as those in the nVNS arm but will not receive any specific intervention from the project. No restrictions will be placed on participants, except that they will be asked not to start any new therapies for IBS or any new medicines unless deemed necessary by their healthcare provider.
- Justification for usual care arm: The use of a sham nVNS device is not feasible in this study due to the current lack of a validated sham nVNS device and the inherent design of the nVNS device, which requires that participants adjust the stimulation intensity until a strong but comfortable tingling sensation is achieved. Due to this need for sensory feedback, blinding participants to the treatment condition is difficult, and a sham device could introduce bias. The Usual Care comparator provides a pragmatic and clinically relevant control while maintaining the methodological integrity needed to assess nVNS efficacy for IBS and comorbid symptoms. Therefore, blinding participants to treatment would be difficult, and a sham device could introduce bias. The Usual Care comparator provides a pragmatic and clinically relevant control that has been used in other IBS studies while maintaining the methodological integrity needed to assess the effects of nVNS on outcomes.
- Minimum participation requirements: Participants in the Usual Care will be required to complete daily symptom questionnaires at least 5 days per week and weekly symptom questionnaires throughout the 8-week trial. If this requirement is not met, participants will be replaced.
    - Questionnaires: Participants will receive a daily questionnaire to their email which will assess daily symptoms and weekly symptom questionnaires. They

are allowed to miss 2 daily questionnaires each week throughout the entire 8-week period.

8. **Midpoint check-in call:** Completed via phone; 5-10 minutes
    a. 4 weeks into the intervention/ UC period study participants will complete a 5-minute phone call with a study team member. The study team will ask the following questions:
        1. Have you had any recent changes in medication since your last MRI visit?
        2. Have you had any changes in medical history since your last MRI visit?
        3. Have you noticed any changes to your bowel habit? (going more often, less often, less/more diarrhea/constipation)
        4. Have you developed any new GI symptoms?
        5. How would you describe your current level of stress? (i.e. work life stress, family, friends, etc.)
        6. Do you have newly scheduled surgeries or procedures (scheduled for next 2 months) that the study team should be aware of?
        7. Have you been completing the questionnaires in a timely manner? Have you had any issues completing the questionnaires?
        8. nVNS only: How often are you using the nVNS device? What intensity level are you using? Have you had any issues with the device? Has it been difficult for you to keep up with the treatment schedule? (i.e. scheduling it into your day, skipping days due to personal responsibilities, etc.)
        9. Females only: Have you had any missed periods? What was the date of your most recent last menstrual period?

POST-INTERVENTION VISIT (Visit 2): UCLA Westwood; 2.5-3 hours

- This visit will be scheduled within 1 week of completing the 8-week intervention. Participants randomized to the nVNS arm will return the nVNS device. The study coordinator will ensure all study questionnaires and assessments are completed at the visit.
- Participants will complete questionnaires per questionnaire schedule. They will receive a questionnaire link via email 7 days before the in-person "post-intervention" visit.
- **MRI Scan:** Participants will undergo the same set of MRI scans from Visit 1 listed above.
- **Blood draw and stool samples:** Participants will complete the same sample collection as listed above in Visit 1. Procedures will be identical.
- Participants who were originally assigned to the Usual Care arm will be offered the nVNS treatment for 8 weeks. If they accept the nVNS treatment, they will be trained to use the device as described for the nVNS arm, and the data collected during visit 2 will be used as baseline data.
    - Once completing the 8-week nVNS treatment, participants will be required to complete Visit 2 and Visit 3.

4-WEEK FOLLOW-UP (Visit 3): Home; 5-10 minutes
Participants will be asked to complete the IBS-SSS and PHQ-15 questionnaire.

De-identified source documents, including study timepoint checklists, will be stored in each participant's REDCap electronic study record. These documents will support study integrity and accuracy by tracking

completion of required procedures and participant adherence. Study coordinators will complete the checklists during participant visits to confirm that all procedures are completed according to the study protocol. The checklists will be organized by study timepoint: 1) baseline/pre-treatment procedures, including questionnaire completion, sample collection with collection dates and times, HRV analysis, MRI scan SOP completion, randomization, and related instructions such as reviewing nVNS device use with participants; 2) intervention/Usual Care procedures, including monitoring timely questionnaire completion, documenting missed questionnaires, and recording changes in nVNS levels; 3) post-intervention procedures, including questionnaire completion, sample collection with collection dates and times, HRV analysis, MRI scan SOP completion, nVNS distribution, and related instructions for Usual Care participants; and 4) 4-week post-treatment follow-up, including questionnaire completion. All documents will also record any deviations from expected study procedures by the study team or participants.

Participants will follow the following the questionnaire schedule:

Validated Measure	Construct Being Measured	Baseline/ Post-intervention	Daily	Weekly	Week 4	Week 8/End of Treatment
IBS and Other GI Symptom Measures						
Bowel Symptom Questionnaire (BSQ)	Rome V questions about IBS, GI symptom severity	X				
IBS-SSS*	Symptom severity in IBS	X		X		X
Bristol Stool Form Scale in the past 24 hrs	Stool Description	X	X			X
Overall GI symptom severity in the past 24 hrs (0-10 numeric rating scale)	GI symptom severity	X	X			X
Overall abdominal pain severity in the past 24 hrs (0-10 numeric rating scale)	Abdominal pain severity	X	X			X
IBS-QOL	IBS quality of life	X				X
Non-IBS Measures						
CMSI	Bothersome comorbid symptoms	X				
COPC	10 chronic pain conditions	X				
PHQ-15*	Somatic symptom severity	X			X	X

PSS	Current stress levels	X			X	X
IPIP-N	Emotional reactivity	X				
CD-RISC	Resilience	X				
Brief resilience scale	Resilience (ability to bounce back from adversity)	X				
SF-12	Generic quality of life	X				X
HAD	Current depression and anxiety symptoms	X			X	X
M.I.N.I Interview	Psychiatric interview for DSM-V and ICD-10 psychiatric disorders	X				
Medication list	Medication history	X			X	X
Adverse events	Side effect history during treatment intervention				X	X

b) Completion of questionnaires will be tracked via the studies RedCap database. Study team members will be instructed to review questionnaires for completion before participant visits. De-identified source documents, including checklists of what needs to be competed at each study timepoint will be stored in RedCap and used to maintain study integrity and accuracy, and monitor participants' adherence to study procedures.

Data variables. Describe the specific data that will be obtained. Alternatively, a list of the data variables may be uploaded to **BruinIRB**. This should reflect the portion of the research that gathers data or specimens from sources other than the participant.

☐ This is included in a separate scientific protocol uploaded to the IRB application

Validated questionnaires (see questionnaire schedule)

Blood samples:

- Blood samples will be collected for whole-blood SNS–immune gene expression profiling and estrogen and androgen metabolites.
- Gene expression profiling: Total RNA will be isolated from whole-blood samples, converted to cDNA using a high-efficiency mRNA-targeted enzyme system (Lexogen QuantSeq 3' FWD), and sequenced on an Illumina HiSeq 4000 instrument. Assays target >10 million sequencing reads per sample, which will be mapped to the GRCh38 reference human transcriptome using the Spliced Transcripts Alignment to a Reference (STAR) aligner (median mapping rate=93.4%) and quantified as gene-specific reads per million mapped reads.

- **Stool samples:**
  - Stool samples will be collected for shotgun metagenomics, metabolomics, and estrogen and androgen metabolites.
- **Shotgun metagenomics sequencing**: Microbial DNA will be extracted using MAG MAX 96 well-plate kits paired with a Thermo KingFisher (Waltham, MA). DNA will be fragmented and barcoded using the Illumina DNA Prep kit (San Diego, CA). Shotgun libraries will undergo 2×150 sequencing on an Illumina NovaSeq X Plus (25B flowcells, 150×2 bp sequencing) to a target depth of 20 million paired-end sequences per sample. Sequence reads will be processed in KneadData for filtering and then inputted into MetaPhlAn4 for taxonomic identification of species against a large reference database. The data will be processed in HUMAnN3 for bacterial gene annotation and source identification. Gene abundances will be aggregated into pathways based on MetaCyc classifications.
- Microbial and Metabolite Stress Index
- **Metabolomics profiling**: LC-MS/MS–based profiling by Metabolon, Inc will identify metabolites from a library of >10,000 molecules, representing a deep global metabolite coverage that is not easily achieved by individual academic labs.
- Estrogen/androgen metabolites
- HRV measurements
- **Brain imaging:**
  - Preprocessing, quality control, and analysis of imaging data will be performed following established published protocols. For each subject, region-specific neuroimaging-derived phenotypes will be computed using the following computational pipelines to "fingerprint" the structure, function, and architecture of each individual brain.
  - Regions of interest: INS (daINS, vaINS, mINS, pINS), ACC (anterior midcingulate [aMCC], pregenual (p) ACC), hypothalamus, amygdala, prefrontal subregions (dorsomedial prefrontal cortex (dmPFC), ventromedial (vmPFC), dorsolateral (dlPFC), ventrolateral (vlPFC)), default mode network (precuneus), cerebellum, and midbrain and brainstem nuclei (periaqueductal gray (PAG), VTA, LCC, PBN, rostral ventral medulla (RVM), DRN, MRN

4. RECRUITING AND SCREENING PARTICIPANTS

Describe how subjects will be identified, recruited, and screened. Include information about how, when, where, and in what setting these processes will occur. Identify who (by position or role, not name) will approach and recruit subjects, and who (by position or role, not name) will screen them for eligibility.

☒ In Person (direct recruitment)
☐ Letter (describe who will receive below)
☒ Phone
☒ Mychart messaging or equivalent secure communication from a medical records system
☒ Participant pool for which potential research participants have given permission for future contact. (*provide the IRB number(s) for the protocol(s) in which this/these pool(s) was/were created below*)
☐ non-encrypted text messages and/or e-mail
☐ encrypted text messages and/or e-mail (UCLA Health and UCOP instructions)
☒ Advertisements/ Flyers/ Information Sheet/ Internet Postings
☒ Referrals (e.g., referrals from non-investigator healthcare providers, snowball sampling, participants referring other participants, etc.)
☐ Review of publicly available data (*indicate the source below*)
☐ Review of other records (*indicate the source below*)

☐ Other (*describe below*)

☐ Potential study participants are identified from another IRB approved study or IRB approved screening protocol. (*provide the IRB number(s) below*)

IRB 26-1173, 20-0540, 20-1118, 20-0515

Recruitment will be conducted by study team members (i.e. Study Coordinator, student research assistants) via the methods listed above.
Flyers will be posted across UCLA locations (i.e. libraries, bathrooms, bulletin boards).

Will existing records be accessed or obtained for recruiting and screening procedures prior to enrollment?

☐ **No**
☒ **Yes →** Describe the source and contents of the records used and whether it will be retained as part of the study data.

The charts of potential participants will be reviewed in CareConnect to determine eligibility based on the listed inclusion/exclusion criteria. Information that will be reviewed is medical history, diagnoses, medications, and past blood work/test results. If the participant is eligible and enrolls in the study, the information will be retained in the study's encrypted database. However, if the participant does not enroll, the information will not be retained.

Recruitment materials. What materials (if any) will be used to recruit and screen subjects? (u*pload recruitment/screening materials to BruinIRB***)**

Recruitment Materials:

- Flyers
- Emails
  - 'generic' email template will be used to reach out to people who indicate interest via word of mouth, missed phone calls, flyers.
- MyChart Messages

Screening Materials:

- Phone Screening Script

All items listed have been uploaded to BruinIRB

Will you access medical records to identify potential subjects without obtaining consent?

☒ Yes
☐ No
☐ N/A

Relationship with participant population. Do any members of the study team have an existing relationship with the study population(s)?

☐ **No**

☒ **Yes** Describe the nature of the relationship, how the potential undue influence of this relationship will be minimized, and (if applicable) how therapeutic misconception will be minimized in the recruitment and consent process

Participants may be recruited from UCLA clinics. Prior to enrollment, participants will be informed that their participation in the study will not impact their medical treatment.

5. CONSENT FOR STUDY PARTICIPATION

Check all the methods that apply to your consent processes:

☒ Obtain signed consent
(Participant or Legally Authorized Representative)

☐ Obtain signed parental permission

☐ Obtain signed assent from children/adolescents or adults unable to consent

☐ Obtain verbal assent from children/adolescents or adults unable to consent

☐ Obtain consent online*
(Waive written documentation of consent)

☐ Obtain verbal consent*
(Waive written documentation of consent)

☐ Waive consent and/or parental permission*

☐ Waive assent / Assent is not appropriate*

Describe the methods that will be used to **ensure or test the subjects' understanding** of the information during the consent process.

☒ subjects will be asked to paraphrase key information about the research.

☐ other (please specify):

N/A

Consent Process Description. Provide a step-by-step description of the consent process, including:

- Outline the personnel involved, and the methods to be used.
- Indicate the type of setting(s) in which the consent process will be conducted – if the setting is not private, describe the measures to protect confidentiality.
- Describe the measures that will be taken to provide prospective research participants with sufficient opportunity to consider whether or not to participate in the study.
- Specify the length of time subjects are given to decide whether they wish to participate in the study.
- Explain how you will assess whether subjects understand the information conveyed during the consent process.
- If applicable, explain if permission will be obtained from individuals other than parents, and if so, how you will determine that the individual providing permission has the authority to do so.

The study team (i.e. Study Coordinators, Student Research Assistants) will complete a phone screening with potential participants using an approved script. Responses will be reviewed and discussed with the PI if necessary. If participants are deemed eligible, the study team will send the Informed Consent Form (ICF), HIPAA Research Authorization Form, and Bill of Rights to the participant via DocuSign.

Participants will be provided with ample time to review the documents and answer any applicable questions. If they remain interested, participants will then be asked to paraphrase key information about the study to confirm they are giving consent to voluntarily participate in the study.

Will the research exclude **subjects who do not speak *English***?

☐ **No**
☒ **Yes**

Please provide a justification for excluding this population

To be enrolled in the study and ensure valid informed consent, participants must be able to understand study procedures, risks, and rights. Non-English speakers are excluded because consent forms and study materials are only available in English, and the study team currently does not have access to qualified interpreters. For example, the UCLA Bowel Symptom Questionnaire (BSQ), is only available in English.

Cultural Considerations. Check all that apply to the population(s) with which this study will be conducted.

☐ Participants may be illiterate or insufficiently literate to be able to comprehend a conventional written informed consent form.
☐ The participants may be reluctant or unwilling to sign a written informed consent form.
☐ The husbands make decisions for their wives.
☐ Elders make decisions for younger adult family members.
☐ Elders make decisions for their community.
☐ It is considered impolite to refuse a request.
☐ People are fearful of refusing requests that they regard as coming from authorities.
☒ **None of the above are applicable to this study.**

If any of the above items are applicable to this study, indicate the steps that you will take to ensure voluntary participation after providing the study information, and if applicable, any planned involvement with the community regarding the consent process.

N/A

Consent-related materials. Upload to **BruinIRB** all consent scripts/talking points, consent forms, debriefing statements, Information Statements, parental permission forms, and any other consent related materials that will be used.

6. INFORMATION ABOUT THE STUDY DATA

Data related to health or mental health care. Check all that apply.

☒ Obtained from a medical or clinical record.
☐ Created or collected as part of health or mental health care.
☐ Used to make healthcare or mental healthcare decisions and/or provided to other healthcare professionals.
☐ Research data will be entered into the participants' medical or clinical record.
☐ **None of the above.**

Indicate all identifiers that may be **accessed or included in the research records** for the study:

☒ Names	☐Health Plan Numbers
☒ Dates	☐ Account Numbers
☒ Age (if over 89 years)	☐ License/Certificate Numbers
☒ Postal Address	☐ Web URLS
☒ Phone Numbers	☐ IP Address Numbers
☐ Fax Numbers	☐ Biometric Identifiers (including finger and voice prints)
☒ Email Address	☐ Facial Photos/Images
☒ Social Security Number*	☐ Any Other Unique Identifier (this does not include the code assigned by the investigator to identify the data)
☒ Medical Record Number	

None of the above ☐

***If social security numbers will be collected** explain why they are necessary, how they will be used, how they will be protected and how long they will be retained.*

For processing of participant stipends, the Department of Medicine (DOM) Finance office may collect SSN.

For research involving biospecimens: Will the research include **whole genome sequencing**?

☒ No
☐ Yes

7. DATA SECURITY

Do you have a data security plan for this study? (check all that apply)

☐ No, I don't have a data security plan, as all research data is collected, stored, and analyzed in analog form.

☒ Yes, I plan to follow the OHRPP Guidance and Procedure: Data Security in Research Guidance

☐ Yes, I have an alternative, equally effective plan (please upload document **or** describe below)

N/A

☒ **In event of data security breach,** I will notify the IRB and my department's IT Compliance Coordinator. See UCLA Policy 420 for more info.

8. PRIVACY AND CONFIDENTIALITY

How will the investigator maintain privacy in the research setting(s)?

All research communication and appointments will be confidential, in private settings. During phone calls, participants will be informed that sensitive data may be asked during the call (e.g. birthdate, medical history).

If the protocol will collect and maintain identifiable data, **explain how the planned safeguards to maintain confidentiality of identifiable data and data security are appropriate** to the degree of risk from disclosure.

To ensure participant confidentiality, all coded data will be stored on password-protected computers, and any paper records will be stored in locked cabinets to prevent unauthorized access. Personal identifiers (e.g., names, contact information) will be separated from study data and stored in a separate, securely protected file. Data files containing coded information will not include personal identifiers unless required for follow-up or safety monitoring, in which case the identifiers will be accessed only by authorized study personnel.

Indicate how personally identifiable information (PII) will be managed during conduct of the study (select all that apply):

☐ Personally identifiable information will be removed and destroyed
☒ The data and/or specimens will be coded
☐ Personally identifiable information will be maintained with the data and/or specimens

If the PII will be removed or the data will be coded, indicate the ***process used for removing PII or coding the data*** and ***who will perform the task***:

Upon enrollment, each participant will be assigned a unique study identification number (ID). This ID will be used to link the participant's data to their records without directly revealing their personal information. The study ID will replace any personally identifiable information (e.g., name, date of birth) in the data collected during the study, including survey responses, biological samples, and other research data.

Will coded or personally identifiable data be collected, transmitted, or stored via the internet/apps?

☐ No
☒ Yes

If yes, indicate all that apply:

☒ A mechanism such as survey monkey, zoomerang, or an e-mail anonymizing service will be used to strip off the IP addresses for data submitted via e-mail.
☒ The data will be encrypted.
☐ A firewall will be used to protect the research computer from unauthorized access.
☐ Controlled access privileges will be used on the hardware storing the data.
☐ Other (please specify):

N/A

***During the study*, indicate how coded data will be stored and secured including paper records, electronic files, audio/video tapes, specimens. Specify how the code key will be securely maintained** (check all that apply)

☐ Not applicable (research data and specimens will not be coded)

Electronic Data:

☒ Encryption or password protection software will be used.

☒ Secure network server will be used to store data.

☐ stand-alone desktop computer will be used to store data (not connected to the server/internet)

☐ A contracted outside vendor will store the code key. The vendor will have a business associate agreement with UCLA.

☐ Other (please specify):

N/A

Hardcopy data, recordings and specimens:

☒ locked file cabinet or locked room with limited access by authorized personnel

☒ locked lab/refrigerator/freezer with limited access by authorized personnel

☐ The code key will be kept in a locked file in a locked room.

☐ The coded data and/or specimens will be maintained in a different room from the code key.

☐ Other (please specify):

N/A

***After the study*, indicate how the data codes and/or personal identifying information will be handled** (check all that apply)

☐ All data files will be stripped of personal identifiers and/or the key to the code destroyed.

☐ All specimens will be stripped of personal identifiers and/or the key to the code destroyed.

☐ Audio or video recordings will be transcribed and then destroyed or modified to eliminate the possibility that study participants could be identified.

☐ Photos or images will be modified to eliminate the possibility that study participants could be identified.

☐ Restricted use data will be destroyed or returned to the source.

☒ Personal identifiers and/or codes linking the data and/or specimens to personal identifiers will be maintained for future research.

(for identified or coded research records that will be maintained for future research) **Provide the following information: 1) how the information/specimens will be securely handled and stored, 2) how will you assure confidentiality, and 3) who will have access to the identifiers and/or codes:**

After the study, information/specimens will continue to be stored in the same manner as described above. The code key linking study IDs to participant identifiers will be kept separate from research data and stored securely. Access to the code key will be restricted to the Principal Investigator (PI), study coordinator, and other designated research personnel who require access to this information for specific tasks (e.g., resolving issues with data or samples, or responding to participant inquiries).

Describe any additional steps, if any, to be taken to assure that the subjects' identities and any personal identifying information are kept confidential:

N/A

State mandated reporting. Each state has reporting laws that require some types of individuals to report some kinds of abuse (such as child or elder), and certain infectious diseases (such as HIV, TB) that are under public health surveillance.

Are you, or a member of the research team, likely to learn of any reportable information while conducting the research **AND** are obligated to report it to state authorities?

☒ **No**

☐ **Yes** → The UCLA IRB expects subjects to be informed of this possibility unless you provide a rationale for not doing so. ***If you will not include these types of disclosures***, please provide your rational:

N/A

Certificates of Confidentiality. Is/will the study be covered by a Federal Certificate of Confidentiality (or equivalent protections) for the research data? *If/when a certificate is obtained, it should be uploaded to* ***BruinIRB****.*

☐ **No**

☒ **Yes,** because the research is *automatically* granted a Certificate by the NIH or the CDC as a condition of the award or an equivalent protection as a condition of funding from another Federal agency.

☐ **Yes,** because I will apply for a Certificate of Confidentiality

9. DATA AND/OR SPECIMENS FOR FUTURE RESEARCH

Possible secondary use or sharing of information, specimens, or subject contact information. Is it likely that the obtained or collected information, specimens, or subject contact information will be used for any of the following:

- Future research not described in this application (in other words, secondary research)

- Submission to a repository, registry, or database managed by the study team, colleagues, or others for research purposes
- Sharing with others for their own research

Please consider the broadest possible future plans and whether consent will be obtained now from the subjects for future sharing or research uses (which it may not be possible to describe in detail at this time). Answer **YES** even if future sharing or uses will use de-identified information or specimens. Consider requirements from Federal funding agencies.

☐ **No**
☒ **Yes** *Answer the questions below:*

Describe **what will be stored for future use**, including whether any direct or indirect (e.g., subject codes) identifiers will be stored.

Once the study data is collected, all samples and analyses will be labeled with a unique study code. This process ensures that personal identifying information is not included in the dataset used for analysis or shared with external researchers.

- Collected biosamples (i.e. stool, tissue, and blood) will be stored in a locked freezer for future analysis. Sample containers will only include de-identified information. If the study investigators wish to share samples in the future, all shared data will be de-identified.

All study information will be maintained in an encrypted file and stored in a secure manner to ensure that unauthorized personnel cannot access it. Any information provided to other researchers or external parties per the PI's discretion will be coded and will not contain any information that can identify a participant. The safety measures outline in Item 8 above (Privacy and Confidentiality) will continue to be used after the study to ensure that all information is kept confidential.

Describe **what will be shared with other researchers or with a repository/database/registry**, including whether direct identifiers will be shared and (for specimens) what data will be released with the specimens.

Any research records that will be shared with authorized, non-UCLA personnel will not contain any identifiable information. All data shared will be coded to ensure that confidentiality is maintained. Any publications or presentations resulting from the study will also be coded.

Employees of the University may have access to identifiable information as part of routine research processing such as lab work or payment processing. These employees are bound by strict confidentiality agreements and are prohibited from disclosing identifiable information.

Who will **oversee and/or manage the sharing**?

The study team will retain oversight of all distributed data and specimens. Periodic checks may be performed to ensure compliance with the agreed upon terms of use, ethical guidelines, and legal regulations.

Researchers will be required to report the outcomes of their studies to the PI or governing body, as applicable, to maintain transparency in the use of data.

What are the distribution rules? (what are the criteria used to determine the adequacy of requests to obtain data and/or specimens – e.g. the type of researchers that will be eligible to receive data or the type of research to be conducted)

- **Research Purpose:** Data will only be distributed to researchers conducting studies that align with the original research goals or that are ethically consistent with the study's objectives. This may include follow-up studies, secondary analyses, or collaborative research initiatives related to the study's focus.
- **Research Proposal Review:** All requests for data will be reviewed by the study's Principal Investigator (PI) or a designated review committee to ensure that the request aligns with the study's original aims and ethical guidelines.
- **Data/Specimen Use:** The requested data must be used for de-identified research. Researchers will be required to submit a detailed proposal outlining the study's purpose, methodology, and how the data will be used.
- **Ethical Approval:** Researchers must have appropriate ethical approval from their institution's IRB (Institutional Review Board) or equivalent body, ensuring the protection of participant privacy and compliance with all relevant regulations.
- **Confidentiality Agreements:** Researchers who request and receive access to study data must sign a confidentiality agreement to ensure that they will maintain the confidentiality of the data and will not attempt to re-identify individuals.

Describe the possible future uses, including limitations or restrictions (if any) on future uses or users. As stated at the beginning of this question, consider the broadest possible uses.

Possible future uses include:

- Secondary analyses based on results of this study
- New research questions that can be asked based on collected data
- Future contact of subjects to answer more questionnaires/surveys
- Obtain more clinical data from chart review

Future contact with subjects. Is there a plan to retain any contact information for subjects so that they can be contacted in the future?

☐ **No**

☒ **Yes** → Describe the purpose of the future contact, and whether use of the contact information will be limited to the study team; if not, describe who else could be provided with the contact information. Describe the criteria for approving requests for the information.

If the study PIs wish to contact study subjects in the future contact may include contact of subjects to answer more questionnaires/surveys for other research questions. This will be limited to any of the principal investigators.

10. RISK/BENEFIT ASSESSMENT

Describe the reasonably foreseeable risks **of harm, discomforts, and hazards to the subjects and others** of the research procedures. For each harm, discomfort, or hazard:

- Describe the magnitude, probability, duration, and/or reversibility of the harm, discomfort, or hazard, AND
- Describe how the risks will be reduced or managed. Do not describe data security protections here, these are already described above.

1. **Potential risk of discomfort due to:**
    a. **Stool and Urine Collection:** Participants may experience mild discomfort, however, it is expected to resolve once they complete their collection.
    b. **Answering questionnaires and providing medical history:** Participants may experience mild discomfort, however, they will be informed that they do not have to answer any questions they do not wish to and can stop at any time.
        i. If participants feel anxious or depressed, participants will be informed to contact their healthcare provider.
    c. **nVNS Stimulation**: Risks of nVNS are expected to be mild and temporary. Any side effects of the application typically resolve immediately after the stimulation is completed.
        i. Participants will be trained to use the nVNS device to ensure correct operation and the study team will be accessible to the participant if clarification is needed.
    d. **MRI**: Participants may experience mild discomfort while being in the scanner, however any effects will typically resolve once the scan is complete.
        i. To minimize discomfort, participants will be provided detailed instructions prior to imaging, offered ear protection, and an emergency squeeze ball to discontinue the study at any time. Additionally, all staff are certified and trained on MRI-related procedures to ensure participant safety.
    e. **Blood Draw:** Risks are intended to be minimal and may include temporary discomfort or pain at the needle insertion site. Risks will be minimized by having trained personnel draw blood using sterile techniques.
2. Participants will be monitored throughout and after the blood draw to ensure their safety.

Are there any research procedures that may have **risks that are currently unforeseeable**?

☒ **No**

☐ **Yes** → Identify the procedures.

N/A

What is your assessment of the risk level of all *research* procedures? (select all that apply)

☒ Minimal risk
☐ More than minimal risk

If more than one category is selected, please describe which response applies to which cohort:

N/A

Anticipated direct benefits to participants. If there are any direct research-related benefits that some or all individual participants are likely to experience from taking part in the research, describe them below:

Participants may or may not benefit directly. Those assigned to nVNS may experience improvement in IBS or other physical symptoms, but the effectiveness of nVNS for this indication is being studied and benefit is not guaranteed. Usual Care participants are not expected to receive a direct benefit during the randomized phase beyond study assessments and monitoring.

Benefits to society. Describe the potential benefits to society including the importance of the knowledge to be gained.

The study may clarify whether nVNS improves IBS and comorbid physical symptoms, identify peripheral and brain-based predictors and mechanisms of response, and determine whether treatment effects differ by sex. This knowledge may support development of more effective, biologically informed, and personalized treatments for IBS.

Potential risks/discomforts. Indicate potential risks/discomforts, if any, associated with each intervention or research procedure, and discuss any measure that will be taken to minimize risks.

- A risk/discomfort is a potential harm associated with the research that a reasonable person would consider important in deciding whether to participate in the research.
- Risks can generally be categorized as physical, psychological, sociological, economic, and legal.
- The information provided should be reflected in risks section of the informed consent documents.
- *If there are no anticipated risks or discomforts, indicate n/a.*

Potential risks associated with blood draws
Blood will be taken from a subcutaneous vein by trained personnel using universal precautions. Blood draws, while generally safe, can carry certain risks that you should be aware of:

- **Discomfort or Pain**: During the insertion of the needle into the vein, you may experience temporary discomfort, bruising, or soreness at the puncture site. This discomfort typically resolves quickly.
- **Bleeding:** After the blood draw, it's possible to experience minor bleeding or hematoma (a localized collection of blood outside the blood vessels) at the site where the needle was inserted. Most bleeding stops on its own or can be controlled with gentle pressure and a bandage.

- **Infection**: Although rare, there is a small risk of infection at the puncture site. Proper sterilization techniques and using sterile equipment reduce this risk significantly.
- **Fainting or Dizziness:** Some individuals may feel faint or dizzy during or after the blood draw, especially if they are sensitive to needles or have a fear of blood. It's important to inform the phlebotomist if you have a history of feeling faint during blood draws.
- **Bruising:** Occasionally, the blood draw can cause a hematoma (a collection of blood that looks like a bruise) under the skin. This is usually harmless and resolves on its own.
- **Vasovagal Response:** Some participants may experience a vasovagal response, which can cause symptoms such as sweating, lightheadedness, or even fainting due to a sudden drop in heart rate and blood pressure. This response is more common in those with a history of anxiety or fainting during medical procedures.

Potential risk of discomfort due to:

- **Potential risks associated with nVNS stimulation:** Participants may experience the side effects listed below. However, these symptoms typically resolve immediately after the stimulation is completed.
    - Application site discomfort, irritation, and/or redness
    - Local pain at the face, head, and/or neck area (including toothache)
    - Muscle twitching and/or contractions (can cause facial droop and/or lip pull)
    - Tingling, prickling, or feeling of 'pins and needles' on the skin where the device is applied (Paresthesia/Dysesthesia)
    - Headache, migraine, dizziness

Potential risk of anxiety or stress due to:

- **Stool Collection:** The collection of stool samples may be inconvenient and cause some discomfort. However, the collection of these samples will be done at your own convenience (i.e., in your own home) to minimize any discomfort.
- **Urine Collection:** The collection of urine in a clinical setting may cause you to feel uncomfortable or anxious. The research staff will provide a private and comfortable environment to collect urine samples to minimizing potential discomfort.
- **Answering questionnaires and providing detailed medical history:** Filling out questionnaires may be inconvenient and uncomfortable. Participants will be informed that they do not have to answer any questions they do not wish to answer and may stop at any time.
- **MRI**: Participants may feel confined or claustrophobic in the scanner, bothered by noise levels while scans are running, and/or feel uncomfortable while lying down for an extended period. However, participants will be given an emergency squeeze ball to end the scan at any time, provided with ear protection, and all information will be thoroughly outlined to participants before beginning.

Privacy and Confidentiality Risks

- **Confidentiality of Biological Samples:** While all biospecimens will be anonymized (assigned a unique identification number rather than personal identifiers) and stored in a secure database, there is a minimal risk of confidentiality breaches due to mishandling or unauthorized access to the research data.
- **Genetic Information:** If genetic testing is performed on any biospecimen, there may be incidental findings that could have implications for participants or their family members. Such findings may relate to genetic conditions or predispositions, though this is rare. While human DNA/RNA may be sequenced as a result of testing, the study team will not analyze the human DNA/RNA.

Risk/Benefit Analysis: Indicate how the *risks to the participants are reasonable in relation to anticipated benefits,* if any, to participants and the importance of the knowledge that may reasonably be expected to result from the study.

The foreseeable risks are primarily mild, temporary, and minimized through eligibility screening, standardized MRI and biospecimen procedures, device training, ongoing symptom and adverse-event monitoring, and access to the study team. Although individual benefit is uncertain, the study addresses an important clinical need by testing a non-pharmacologic treatment for IBS and evaluating biologic and brain-based predictors of response. The risks are reasonable in relation to the potential scientific and clinical value of the knowledge to be gained.

11. DATA AND SAFETY MONITORING

Is there a separate Data Safety Monitoring Plan?

☒ **No**
☐ **Yes** (please upload to the application)

Indicate who will be **responsible for overseeing study safety** (check all that apply)?

☒ The **Principal Investigator**
☐ A **designee** of the Principal Investigator
☐ A formally constituted **Data Safety Monitoring Board** (DSMB/DMC).
☐ A **medical monitor** designated by the Sponsor
☐ **Other** (please specify)

N/A

Are there plans for an **interim analysis?**

☒ **No**
☐ **Yes,** and the plans are described in a separate scientific protocol uploaded to the IRB application.
☐ **Yes** (please describe)

N/A

Have **stopping rules** been established for this study**?**

☒ **No**
☐ **Yes,** and the stopping rules are described in a separate scientific protocol uploaded to the IRB application.
☐ **Yes** (please describe)

N/A

Are there **defined rules for withdrawing participants** from study interventions**?**

☐ **Not applicable**
☐ **No**
☐ **Yes,** and the defined rules are described in a separate scientific protocol uploaded to the IRB application
☒ **Yes** (please describe)

Participants may stop the intervention or withdraw at any time. The investigator may discontinue the intervention or withdraw a participant for a serious or intolerable adverse event; a new medical condition or medication that creates a safety concern or substantially affects study interpretation; pregnancy; an MRI contraindication; inability to use the device safely; repeated failure to follow essential study procedures; or a determination that continued participation is not in the participant's best interest. Starting antibiotics, probiotics, or a new IBS-directed therapy will be documented and may result in discontinuation of the intervention or exclusion from per-protocol analyses, but participants will not automatically be removed from all follow-up unless safety or data integrity requires it. Whenever feasible, participants who discontinue the intervention will be asked to complete safety follow-up and outcome assessments.

Un-blinding. If this is a double-blinded or single-blinded study in which the participant and/or relevant study team members do not know the group to which the participant is assigned, describe the circumstances under which un-blinding would be necessary, and to whom the un-blinded information would be provided.

☒ **N/A**
☐ The circumstances for un-blinding are described in a separate scientific protocol uploaded to the IRB application.

N/A

Is it anticipated that the research will produce any **individual research results** that are **clinically actionable**? (select all that apply)

☐ **No,** because there are serious questions regarding the validity or reliability of the research results
☐ **No,** because the return of results has the potential to cause bias
☒ **Yes** → Answer the following questions

Describe the **clinically actionable results that are anticipated** and explain which results, if any, could be urgent (i.e. because they pose life-threatening or severe health consequences if not treated or addressed quickly).

The full MRI scan is not provided to participants. However, if the MRI personnel identify any abnormalities during the scan, it will be deferred to the radiologist on site for review.

All participants who complete a CBC, Celiac Serology, C-reactive protein levels, and/or Fecal Calprotectin will have results viewable in their charts.

Explain which of these results will be offered to subjects.

If the radiologist deems appropriate, the participant will be provided with a brain image and informed to follow up with their healthcare team.

All test results listed above will be visible in participants' MyChart portals. If any of the results are abnormal, they will be advised to follow-up with their healthcare team.

Explain which results will not be offered to subjects and provide the rationale for not offering these results.

All other data collected during the study will not be offered to subjects because the results will not be analyzed in real time. Additionally, all data will be deidentified at the time of analysis.

Is there a plan for offering subjects any **results that are not clinically actionable**?

☒ **No**

☐ **Yes** → Explain which results will be offered to subjects and provide the rationale for offering these results.

N/A

Describe the **validity and reliability of any results that will be offered** to subjects.

N/A

Describe the process for **communicating results to subjects** and facilitating understanding of the results. In the description, include who will approach the participant with regard to the offer of results, who will communicate the result (if different), the circumstances, timing, and communication methods that will be used.

In situations where the study team identifies abnormal results, they will immediately inform the participant and recommend them to follow up with their healthcare team. They will also be provided with the study PI's contact information if they have further questions.

If a participant does not have a physician, the study team will assist them in finding a physician to follow up with.

Describe any plans to **share results with family members** (e.g. in the event a subject becomes incapacitated or deceased and the results could impact the subject or other blood relatives).

N/A

12. STATISTICAL PLAN

Describe the **proposed statistical procedures or descriptive analyses** for the study**.** If applicable, **indicate how the sample size was determined** and **the definition(s) of outcome endpoint(s).**

☐ The statistical plan is described in a separate scientific protocol uploaded to the IRB application.

Analyses will follow the intention-to-treat principle, with prespecified per-protocol sensitivity analyses based on adherence. Linear mixed-effects models will compare nVNS and Usual Care for repeated IBS-SSS and PHQ-15 measurements at baseline and Weeks 4, 8, and 12. Models will include treatment, time, treatment-by-time interaction, sex, and relevant baseline covariates, with participant-specific random effects. The primary treatment comparison is the between-arm difference in change from baseline to Week 8. Durability will be assessed at Week 12. Logistic regression will compare the proportion of IBS-SSS responders (≥50-point reduction) at Week 8. Exploratory treatment-by-sex-by-time interactions will estimate sex-related differences. Moderator models will test whether baseline SR score and peripheral biologic measures modify treatment response through SR-measure-by-treatment-by-time interactions. Changes in peripheral biologic measures will also be compared between groups and correlated with symptom change.

BRAIN IMAGING:
The GLM will be used to identify changes in the SR-related brain phenotype between baseline and the 8-week nVNS intervention compared to Usual Care. Pre-treatment and post-treatment contrast (task) or correlational maps (task-free) will serve as dependent variables for second-level analyses. GLM will be applied to correlate changes in the SR-related brain phenotype with changes in clinical symptoms and peripheral biologic measures of SR controlling for covariates. We will use moderator analyses with the framework logistic regression and GLMs to test whether higher scores on the SR brain phenotype predict response (≥50-point reduction in IBS-SSS, improvements in extraintestinal symptom severity (PHQ-15)) to nVNS compared to the Usual Care treatment arm. We will specify models with the main effects for group (F nVNS, M nVNS, F Usual Care, Male Usual Care), SR brain phenotype, and their interaction along with covariates. amine significant SR brainphenotype×treatment interaction effects, we use graphical methods and estimate the parameter estimates(betas reflecting the association between the SR brain phenotype scores and the dependent variables at high(+1 SD), moderate (mean), and low (−1 SD) levels of the SR brain phenotype scores along with 95% CIs for two treatment arms. LCA using single-degree-of-freedom simple-slopes analysis tests on the parameter estimates to test for nVMS v Usual Care difference in the association between the SR brain phenotype and treatment response. Durability will be assessed at Week 12. Data collected during the delayed-treatment extension and after Week 8 will not be included in the primary efficacy analysis and will instead be used for exploratory analyses. Logistic regression will compare the proportion of IBS-SSS responders (≥50-point reduction) at Week 8. Exploratory treatment-by-sex-by-time interactions will estimate sex-related differences. Moderator models will test whether baseline SR score and peripheral biologic measures modify treatment response through SR-measure-by-treatment-by-time interactions. Changes in peripheral biologic measures will also be compared between groups and correlated with symptom change.

The primary imaging analysis will compare baseline-to-Week-8 change in prespecified SR-related brain phenotypes between nVNS and Usual Care using general linear or mixed-effects models with appropriate covariates and correction for multiple comparisons. Secondary analyses will test associations between imaging changes and changes in IBS-SSS, PHQ-15, heart-rate variability, SNS-immune gene expression, microbial/metabolite stress indices, and sex-hormone measures. Baseline brain signatures will be evaluated as

predictors of clinical response. Exploratory models will test sex-by-treatment effects and whether females with greater baseline comorbid symptom burden show larger brain changes.

As alternative strategies, PLS and/or PALM will be utilized to evaluate similarities and differences in connectivity changes (post-treatment – pre-treatment) between groups, FSL, as well as correlations between changes in symptom severity, SR, comorbidities, and connectivity changes. Corrected p-values will be determined by boot-strapping (5000 bootstraps) and permutations (5000 permutations) for PLS and PALM, respectively. We will also employ sPLS-DA and sPLS-R algorithms to determine baseline brain signatures that predict a change in IBS-SSS and change in comorbid symptom severity (PHQ-15). The primary imaging analysis will compare baseline-to-Week-8 change in prespecified SR-related brain phenotypes between nVNS and Usual Care using general linear or mixed-effects models with appropriate covariates and correction for multiple comparisons. Secondary analyses will test associations between imaging changes and changes in IBS-SSS, PHQ-15, heart-rate variability, SNS-immune gene expression, microbial/metabolite stress indices, and sex-hormone measures. Baseline brain signatures will be evaluated as predictors of clinical response. Exploratory models will test sex-by-treatment effects and whether females with greater baseline comorbid symptom burden show larger brain changes.

ADDITIONAL CLINICAL DATA:
Briefly, we will apply an intent-to-treat analysis. GLMs will be applied to compare the nVNS and Usual Care arms for primary efficacy endpoints, changes in IBS symptom severity (as measured by IBS-SSS) and comorbid symptom scores (as measured by) PHQ-15 scores at 8 weeks relative to baseline). We will also assess the durability of effects at 12 weeks. IBS-SSS or PHQ-15 scores will be the dependent variables. The model will include the treatment arm (nVNS vs. Usual Care), time (baseline, Weeks 4, 8, and 12), and their interactions as fixed effects and subject as a random effect. We will include model-specific covariates (i.e., BMI, Hospital Anxiety and Depression scores, diet, alcohol, smoking, hypertension, diseases that affect ANS, medications, and caffeine intake). In a secondary analysis, we will apply logistic regression to test whether the IBS-SSS responder rate is higher in the nVNS group than in the Usual Care group. In secondary exploratory analyses, we will apply GLMs to investigate potential sex differences in treatment effects (sex×treatment×time) and generate effect size estimates for future work. We will apply moderator analysis using GLMs to determine if the baseline SR composite score and biologic measures of SR influence treatment response. IBS-SSS or PHQ-15 scores will be the dependent variable. The model will include the main effects for SR measures (e.g., HRV), treatment arm (nVNS vs Usual Care), time (baseline, Weeks 4, 8, and 12), and their interactions and a random effect for subject. A three-way interaction (SR measure×group×time) will suggest that SR measure moderates the response to treatment over time. The primary hypotheses will be tested via a single-degree-of-freedom simple slopes analysis test contrasting the parameter estimates between the two treatment arms (nVNS vs. Usual Care) at low (−1 SD), moderate (mean), and high (+1 SD) levels of SR measures at Week 8. We will test the efficacy of nVNS in those who complete Usual Care but choose to receive nVNS treatment for 8 weeks by testing within-group changes in IBS-SSS and PHQ-15 scores. We will compare within-group changes to changes observed in the original nVNS treatment group.

Sample size: Our power calculations are based on a final evaluable sample of 200 participants with IBS who will complete the study (N=100 in each arm, 50 females and 50 males). We anticipate a 15% dropout and a 15% screenfail rate and will enroll additional participants necessary to ensure we achieve this target of 200 evaluable participants. The study was powered for efficacy in response to treatment. Although the primary efficacy analyses will utilize a longitudinal mixed-effects model (including treatment, time, treatment-by-time interaction, covariates, and a participant-level random effect), the power calculations were performed using two-sample t-tests and GLMs as conservative approximations. Because we currently lack the specific pilot data

necessary to accurately estimate the longitudinal covariance structure required for a mixed-model simulation, these cross-sectional approximations provide the most reliable baseline. Additionally, because mixed-effects models account for intra-subject correlation across repeated measures, they are mathematically more efficient and generally provide equal or greater statistical power than their cross-sectional counterparts making our sample size estimation more conservative. Therefore, we powered the analyses based on the two-tailed, single-degree-of freedom test used to test for reductions in our two primary outcomes: IBS severity (IBS-SSS) and comorbid symptom scores (PHQ-15) at 8 weeks in the nVNS arm compared with the Usual Care arm. Based on an alpha=0.025 (0.05/2, for 2 primary outcomes), 100 participants per treatment arm will provide adequate power (80%) to detect a significant effect as small as Cohen's d=0.44, t(198)=2.25 (ncp=3.10, one-tailed t-test). The effect sizes achieving significance ranged from d=0.60 to 1.0 in similar studies, suggesting that these effect sizes are reasonable. We will also estimate effect sizes for sex differences between the treatment arms. Based on our sample size (N=50 M and 50 F per treatment arm), we would be able to detect an effect size (IBS F>IBS M) as small as Cohen's d=0.57, t(98)=1.98, ncp=2.83, alpha=.025. G*Power was used to estimate the power to detect a significant 3-way interaction term (e.g., HRV×treatment×time) in the GLM that includes the largest number of predictors based on a multiple regression model with a total of 16 predictors (including 3 main effects for moderator, treatment, and time, 3 two-way interactions, and 1 three-way interaction of biological measure of SR, treatment, and time, and 9 covariates [e.g., BMI, HADS, diet, alcohol, smoking, hypertension, diseases that affect ANS (e.g., POTS), medications (betablockers, anti-depressants, ADHD meds], caffeine intake)]. Based on an alpha =0.01 (0.05/5, for 5 moderators: composite score, SNS–immune gene expression, HRV, microbial stress index, and metabolite stress index) of 200 participants will provide adequate power (80%) to detect a significant effect as small as f2=0.14, r=.35, F(1,183)=2.10,ncp=28.36. For the two-tailed single-degree-of-freedom tests comparing parameter estimates (at high, moderate, or low levels of the moderator variable) between nVNS (n=100) and Usual Care (n=100) [H2] at alpha=0.0167(.05/3 tests), we would have 80% power to detect effect sizes as low as Cohen's d=0.44, t(198)= 2.26, ncp=3.10. The effect sizes achieving significance ranged from d=0.60 to 1.0, in similar studies, suggesting that these effect sizes are reasonable. Additionally, for analysis of the difference in IBS-SSS responder rate (≥50-point reduction in IBS-SSS) by treatment, at alpha=0.05, we have 80% power to detect a difference in response of 17% (Fisher's exact test).

This study was powered to assess the primary outcome, the difference in pre–post-treatment change in SR related brain phenotypes (structural, connectivity, activity) identified in Aim 2 between the nVNS and Usual Care treatment arms (H1). Using an FDR-corrected two-sided alpha level of 0.0047 (assuming the null hypothesis is false in 10% of hypotheses), a sample size of 200 (nVNS arm: 50 M and 50 F; Usual Care arm: 50 M and 50 F) would provide 80% power to detect an effect-size difference as small as Cohen's d=0.52, t(198)=2.86, ncp=3.71. For testing associations between changes in scores on the SR-related brain phenotype signatures and changes in IBS severity, comorbid symptom scores, and peripheral biologic measures of SR (H2), 200 participants provides 80% detect an effect size association, r>=.25, f2=07, t(198)= 2.86,ncp=3.71, alpha=0.0047, two-tailed test. For the GLM testing whether to higher scores on the SR brain phenotypes identified in Aim 2 predict response (Pre-Post change in extraintestinal symptoms) in nVNS compared to Usual Care (H3), power analyses based on a multiple regression model with 6 predictors (Baseline SR brain phenotype scores, treatment, Baseline SR brain phenotype scores× treatment , and 3 covariates), we have the power to detect a significant Baseline SR brain phenotype scores× treatment as small r>=.25, f2=07, t(198)= 2.86,ncp=3.71, alpha=0.0047, two-tailed test. For the two-tailed single-degree-of-freedom tests comparing parameter estimates (at high, moderate, or low levels of the SR brain phenotypes) between nVNS (n=100) and Usual Care (n=100) at alpha=0.0167(.05/3 tests), we would have 80% power to detect effect sizes as low as Cohen's d=0.44, t(198)= 2.26,ncp=3.10.

Additionally, for analysis of the difference in responder rate (≥50-point reduction in IBS-SSS) by treatment, at

alpha=0.05, we have 80% power to detect a difference in response of 17% (z=1.64) [H3].
For exploratory analyses(H4) testing whether females with IBS with higher comorbid symptom scores will present with greater changes in SR-related brain signatures following the nVNS intervention compared to male IBS, we estimated the power to be based upon a multiple regression model with a total of 6 predictors (comorbid symptom score×group comorbid symptom score×group, as well as main effects for covariates, age, BMI, and diet). Based on n=200 participants, there 80% power to detect a significant moderator effect (comorbid symptom score×group) as small as f2=0.07(r=0.25), F(1,181)=8.19,ncp=13.76. For the two-tailed single-degree-offreedom tests comparing parameter estimates (at high, moderate, or low levels of the comorbid symptom scores) between female (n=50) and male (n=50) IBS at alpha=0.0167(.05/3 tests), we would have 80% power to detect effect sizes as low as Cohen's d=0.66, t(98)= 2.44,ncp=3.28.

For Project 2, Aim 3, the study was powered to the primary outcome, pre-post treatment change in the SRassociated brain phenotypes in nVNS compared to usual care difference in pre-post treatment change in the brain phenotypes alpha =0.0047 a sample size of 200 IBS (nVNS (50 male, 50 female) and usual care (50 male,50 female) would provide and a corrected alpha of 0.0047 provide sufficient power to detect an effect size differences as small as 0. 0.52, if it exists. Exploratory analyes of sex differences in nVNS would have limited power, d=.75

13. FINANCIAL CONSIDERATIONS FOR PARTICIPANTS

Will all protocol-required items and services that produce data for the study be funded by intramural or extramural funding/support?

☒ Yes – **neither subjects nor their insurers will be billed** for any protocol-required items or services (check all that apply

☐ Yes – **we will bill** one or more protocol-required items or services to participants or their insurers (select types)

- ☐ Items and services that are required by the protocol and are **not** part of regular care
- ☐ Items and services that would have been provided to subject even if they were not in the study
- ☐ Health care given during the study as part of regular care
- ☐ Items or services needed to give you study medications or devices
- ☐ Monitoring for side effects or other problems
- ☐ Deductibles or co-pays for these items and/or services

☐ **Not applicable** – this is a non-intervention study (e.g., observational/registry/retrospective study without active treatment) that does **not** require additional visits, labs, items or services performed solely due to study participation

Will **subjects or their insurers be billed for investigational products** (drugs, biologics, devices)?

☐ Yes (attach relevant documentation to support charges including but not limited to FDA and/or CMS approval letter(s) to the IRB application)
☒ No
☐ Not applicable (there is no investigational product in this protocol)

Reimbursements to subjects. Describe any research-related reimbursements to subject, include covering parking costs.

Parking vouchers will be provided to all participants for all in-person visits.

Payment to subjects. Describe any payments made to subjects. If there are multiple study visits for which subjects are paid, please indicate if payments are pro-rated and when payment will be made. List the total payments for research participation.

Screening Visit (if needed): $25

- Blood Draw: $15 x 2
- Stool Sample: $25 x 2
- MRI Scan: $100 x 2
- nVNS, Fitbit, and Questionnaires: $200
    - Questionnaires include all study questionnaires at baseline, daily, weekly, at the end of the intervention, and at 4-week follow up
- Ovulation Tracking: $15

*If participants in Usual Care opt to do the nVNS arm, they will paid the additional amount below:

- Blood Draw: $15
- Stool Sample: $25
- MRI Scan: $100
- nVNS, Fitbit, and Questionnaires: $200

Travel expenses are not reimbursed; however, parking is paid for each in-person visit

14. STUDY TEAM TRAINING AND COMMUNICATIONS

Describe how each study team member will be adequately trained and informed about the research procedures and requirements (including any changes) as well as their research-related duties and functions. Include communication between sites if this is a multi-site study.

☐ There is no study team (the PI will conduct the study without staff)

☒ There are no other sites (besides UCLA)

All study team members have completed and stay up-to-date on all mandatory UCLA trainings for clinical research. Study specific training is conducted by the study team and usage of study SOPs.