Sex related differences in the effect of cognitive behavioral therapy (CBT) on emotional arousal and salience circuits and the role of the gut microbiome Project 3

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Sex related differences in the effect of cognitive behavioral therapy (CBT) on emotional arousal and salience circuits and the role of the gut microbiome Project 3
UCLA IRB 20-001118

Lay Summary:

Cognitive Behavioral Therapy (CBT) is the most well researched and most effective treatment for IBS that targets the brain-gut-microbiome (BGM) axis, and preliminary data show that this therapeutic effect is associated with a reduction of brainstem connectivity with other brain networks and a change in the gut microbes associated with IBS. The increased prevalence of IBS in women (likely related to IBS female's increased sensitivity to a variety of internal and external stimuli) and the higher rate of over lapping non-gastrointestinal pain conditions, suggest the presence of important sex differences in some of these BGM mechanisms.

Research performed by our group during previous SCOR funding has established an increased responsiveness of CRF (corticotrophin releasing factor) and the Locus Coeruleus system (area of the brain that responds to stress and panic) in female IBS subjects, suggesting that this noradrenergic brainstem system plays an important role in IBS pathophysiology.

In addition, our earlier research has begun to identify clinical, functional and cortical brain mechanisms that may underlie these sex effects. Based on these preliminary data, the overall goal of this Project is to use CBT as a probe to study the relationship between specific disease-related alterations of the brain, the gut microbiome, and symptomatic outcomes, and identify the role of sex differences in these relationships.

We will study 150 male and female IBS patients before and after CBT (or usual care) using the advanced

neuroimaging and microbiome technologies in this project. The study will randomized subjects to usual care (33%) or CBT (66 %).

The following is a short summary of this study:

This study length is 12-14 consecutive weeks during which time the subject will be expected to complete visits 1-3 and CBT treatment. Participants will come to the study center for 3 (usual care group) or 7 (CBT group) visits. The first visit is screening (2-3 hours) to ensure eligibility from both a safety and scientific standpoint. Visit 2 is the pre-CBT /Usual care MRI (~3 hours), followed by randomization to CBT or usual care for 10 weeks. If the subject is randomized to the CBT group, they will come to the center for four 1-hour in person cognitive therapy visits. All subjects will return for visit 3, a post-CBT final MRI visit (~3 hours). All subjects will collect a stool and saliva sample at home before and after the usual care or CBT intervention and have a blood sample drawn. Visit 4 will be a 3 month follow up visit post CBT with questionnaires, a blood and stool sample.

There will be several links for on-line at home survey monkey questionnaires to complete, associated with the visits.

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

Cognitive Behavioral Therapy (CBT) is the most well researched and most effective treatment for IBS targeting the brain-gut-microbiome (BGM) axis, and preliminary data show that this therapeutic effect is associated with a reduction of brainstem connectivity with other brain networks.

The increased prevalence of IBS in women, the higher rate of comorbid non-GI pain conditions, as well as the higher prevalence in female
IBS of increased sensitivity to a variety of internal and external stimuli (multisensory sensitivity) suggest the presence of important sex differences in some of these BGM mechanisms.

Research performed by the UCLA SCOR during previous funding has established an increased responsiveness of the CRF-Locus Coeruleus
(LCC) system in female IBS subjects, suggesting that this central noradrenergic brainstem system plays an important role in IBS pathophysiology.

In addition, our earlier research has begun to identify clinical, functional and
structural brain mechanisms that may underlie these sex effects. Based on these preliminary data, the overall goal of this Project is to use CBT as a probe to study the relationship between specific disease-related alterations of the brain, the gut microbiome, and symptomatic outcome, and identify the role of sex differences in these relationships. We will study male and female IBS patients before and after CBT using
the advanced neuroimaging and microbiome technologies of the overall SCOR.
Aim A. will identify sex differences in the impact of CBT on the connectivity between brainstem nuclei and brain networks and between these networks at rest and after provocation.

Aim B. will identify sex differences in the impact of CBT on gut microbiome parameters.

Aim C. will identify clinical, brain and gut microbiome predictors of
CBT outcomes.

Background and Significance: Provide a summary of the background for this study and explain how it will contribute to existing knowledge.

Cognitive Behavioral Therapy (CBT) is the most well researched and most effective treatment for IBS targeting

the brain-gut-microbiome (BGM) axis. Preliminary data show that this therapeutic effect is associated with a reduction of brainstem connectivity with other brain networks and a change in relative abundance of Bacteroides taxa in the gut. The increased prevalence of IBS in women, the higher rate of comorbid non-GI pain conditions, as well as the higher prevalence in female IBS of increased sensitivity to a variety of internal and external stimuli suggests the presence of important sex differences in some of these BGM mechanisms.

Research performed by the UCLA SCOR during previous funding has established an increased responsiveness of the CRF-Locus Coeruleus (emotional arousal system) in females with IBS suggesting that this noradrenergic brainstem system plays an important role in IBS pathophysiology. In addition, our earlier research has begun to identify clinical, functional and brain mechanisms that may underlie these sex effects.

Based on these preliminary data, the overall goal of this Project is to use CBT as a probe to study the relationship between specific disease-related alterations of the brain, brainstem, the gut microbiome, and symptomatic outcome, and identify the role of sex differences in these relationships.

We will study male and female IBS patients before and after CBT or usual care, using advanced neuroimaging and microbiome technologies to test the following:
Aim A: Identify sex differences in the impact of CBT on connectivity between brainstem nuclei and brain networks at rest and after provocation (abdominal threat)
Aim B: Identify sex differences in the impact of CBT on gut microbiome.
Aim C: Identify clinical, brain and gut microbiome predictors of CBT outcomes.

Research Design and Methods: Describe in detail the design and methodology of the study.

A total of 150 adult IBS participants will be completed in years 1-4 of the proposed studies. These will be 50 males, 50 premenopausal females in the treatment arm (Cognitive behavioral therapy: CBT) and 50 in 'usual care' arm, all bowel habit subtypes.

Screening V1: All subjects will have a pre-screening using study specific IRB approved prescreening tool (15-20 minutes). lf eligible and interested we will send the ICF via email to the subject, review it with them and determine their understanding of study participation and risks/benefits. They will sign and return to the coordinator by electronically/scan/fax. We will then send them a short link of preliminary questionnaires via survey Monkey to further determine eligibility for participation and assist the clinician in obtaining accurate medical history as well as assess any risk factors of participation. When determined to be eligible we will set up a video/zoom /phone meeting to complete the initial screening visit/medical and psychological history taking. This will take about 1.5 hour.

They will come to the OCNSR center to complete the study screening, including a modified physical exam by the RN/NP or study team member qualified. If the subject has had a PE with their own PCP and the PI has no concerns, the exam will be waived and just the vital signs, height, weight, and waist circumference is done. At in house screening at arrival, they will initial/date their previously e-signed ICF and have time to ask any questions. The modified physical exam will include vital signs, height, weight, and a ROS (review of systems). There will be a single blood sample taken of about 10cc's (if female) to determine menstrual cycle and if needed for IBS-diarrhea or mixed predominance to rule out any other gastrointestinal disease similar to IBS symptoms there may be additional blood (about 15cc's/1 tbls). They will be given sample collection items to take home for stool and diet diary collection. The stool sample will be collected when they return for the scheduled MRI within 1-3 weeks of screening. They will be given two unique de-identified on-line survey Monkey link to complete sometime before the MRI visit. One link is the Vioscreen comprehensive Diet History Questionnaires and the other is a group of questionnaires asking about symptoms (IBS), mood and general health and wellbeing. This visit will take about 3 hours.

MRI V2: Brain and Brainstem Data Acquisition: All participants will undergo a 60-70 minute structural, resting state, DTI and brain stem MRI scanning session. All subjects will be imaged on a Siemens 3 Tesla Prisma scanner housed at the UCLA Ahmanson-Lovelace Brain Mapping Center (ALBMC). They will complete several state anxiety and symptom measure questionnaires upon arrival as to "how you are feeling right now". Females will need to do daily urine test at home to identify their menstrual phase. When arriving to MRI they will give a urine sample for pregnancy testing and will be excluded if pregnant. A single blood sample will be collected via venipuncture for hormones, cell methylation and pro inflammatory cytokines (15-20 ccs). Subjects will return with their diet diary, stool and saliva (female) sample collections and have reviewed by study coordinator, for viability and collection accuracy.

Scanning will follow parameters of the HBC (Human Brain connectome) project with anterior and posterior views for each scan. They will have the structural MP Rage (gray matter imaging) scan (time ~14.5 minutes) and subjects can watch a movie of their choice during this scan.

Following this is 2 RSN (resting state) functional scan (total time ~12 minutes). Instructions verbatim are given to subject during the RSN is to rest relax and focus on the crosshair displayed on mirrored screen reflected on the head coil, and do not go to sleep. Following the resting state scan the subject will be asked if they had any pain or discomfort anywhere that may have distracted them during the functional resting state and this will be documented on a subject chart.

The next scan is the DTI (diffusion tensor imaging) lasting approximately 10 minutes. Subjects can watch a movie of their choice during this scan.

After scanning completion, subjects will complete the PANAS-SF questionnaire to measure positive and negative emotions post threat in comparison to the pre-threat state. This visit will be no more than 3 hours. Subjects will then be randomized to CBT (66.5 % chance) or usual care (33.5 % chance). The CBT is described below. If usual care the subject will not have any restrictions on them except we will ask them not to start new therapies for IBS or new medicines unless it is deemed necessary by their PCP.

CBT: This structured educational and skills-based intervention is carried out via internet instruction and individual practice. Subjects will be asked to log into the CBT site once per week for 10 weeks with each session lasting between 20 and 40 minutes. The content has been validated in a randomized internet based CBT trial for IBS. The intervention does not entail any direct therapist contact or interaction with other patients or study participants. The intervention is hosted by Mahana Therapeutics Inc. (named Parallel TM) who is contracted by UCLA to provide the intervention. Following enrollment each subject will receive a secure login for accessing the CBT site and no personally identifying information will be asked for or passed along to the site or Mahana Therapeutics. During the 10 week intervention period subjects will be contacted by a study coordinator for follow up should they stop logging in to the web site and they will be able to contact a study coordinator with questions regarding the intervention, website access, etc.

A responder will be defined as a subject with IBS-SS score change of $\geq$50 from baseline to end of treatment and corresponds to a clinically meaningful change in symptoms. Participants assigned to the Usual Care condition will have the same questionnaire, neuroimaging, biological and symptom assessments as those in CBT but will not have any specific intervention from the project.

There may be a short mid study assessment on SM related to symptoms.

MRI Visit 3: After 10 weeks of CBT (within 2-3 weeks of completion) they will return for a post-CBT MRI visit identical to description above. This visit will be no more than 3 hours.

Visit 4: Subjects will be followed at 3 months post CBT to determine if changes were maintained with questionnaire data, blood and stool sample. This visit will take about one hour

If a subject has completed Project 1 (IRB # 20-000540 or Project 2 (IRB #20-000515) associated with this same U54 NIH Grant, and they meet all of the criteria of this project, they will be invited to participate in this CBT/Usual care study. If this occurs, those subjects will not repeat the screening or the pre-CBT MRI visit of this project. They will need to enroll in the study within 3 weeks of the MRI and they will only need to do the CBT/Usual care requirements and the post MRI visit/samples and diet diary. The stipend will be prorated accordingly.

Indicate the specific inclusion criteria for enrollment of each of the groups of research participants in this study.

1. 18 to 55 years of age, inclusive.

2. Healthy control subjects must be without any gastro intestinal history or chronic pain syndromes including a diagnosis or symptoms of Irritable Bowel Syndrome (IBS) or first generation family history of IBS or chronic pain syndrome.

3. IBS subjects meet ROME IV diagnostic criteria for IBS (all sub-types).

4. If IBS, must be absent of red flags including unexplained weight loss, fevers, anemia or blood in stools.

5. All female subjects must be premenopausal menstruating females.

5a. If female and of childbearing potential (i.e., not post-menopausal or documented to be surgically sterile) must be not pregnant, post-partum or breast feeding for at least 6 months. Must have a negative urine test for pregnancy. Must be willing to avoid pregnancy and practice a non-hormonal birth control method such as abstinence, non-hormonal IUD's, or barrier method with spermicide, during the time of study enrollment. Subjects who are on hormonal based birth control (e.g OCP's or implants) will be allowed in the study as long as they still get a monthly menses so menstrual phase can be identified using ovulation kits.

6. Ambulatory outpatient (not depending exclusively on a wheelchair for mobility)

7. Ability to speak the English language fluently due to the standardized assessments involved.

9. Willingness to participate in this study as evidenced by a signed, written informed consent form.

Indicate the specific exclusion criteria for each of the groups of research participants in this study.

1. Evidence of structural abnormality of the gastrointestinal tract. Subjects with IBS must be without 'red flags', that may indicate gastrointestinal disease. Exclusionary GI conditions include but are not limited to gastrointestinal surgery (exceptions: appendectomy, benign polypectomy, cholecystectomy).

2. Presence of a significant and ongoing medical problem that would interfere with participation in the study or testing the study hypotheses (e.g., major heart disease, neurological disorders, inflammatory diseases such as IBD; esophagitis, celiac disease, gastrointestinal malignancy or obstruction, peptic, duodenal or gastric ulcer disease, etc.).

3. Presence of a major psychiatric diagnosis such as schizophrenia, Bipolar disorder, current ADD diagnosis, Post-traumatic Stress Disorder, Obsessive Compulsive disorder or current history of tobacco or alcohol abuse. Subjects with a history of DSMIV diagnosis of Anxiety or Depression in whom symptoms are not active, will be allowed but noted for post-hoc analysis.

4. Use of investigational drugs, products or devices within 28 days prior to screen and through study participation.

5. Subjects with current regular use of narcotics and or opioids. Use of centrally acting medications that will interfere with the neuroimaging testing (such as systemic steroids, opiate analgesics). Medications which alter GI motility and gastric pH will need to be stopped at least 72 hours prior to any physiologic test visit and during the baseline and treatment intervention. For this we will ask the subject to get approval from their own PCP to stop prior to any intervention. However, rescue medication of bisacodyl and loperamide will be allowed for prolonged constipation and diarrhea, respectively, during intervention periods but not during baseline or within 3 days of follow up MRI. For rescue medication, such as laxatives or antidiarrheal, we will ask to hold off use up to 3 days prior to collecting stool sample.

6. Subjects with obesity (BMI >35)

7. Subjects with claustrophobia, metal implants, dental braces, large tattoos (e.g. full arm or back); making MRI safety not possible.

9. Subjects with history of heavy use of tobacco products or current use of more than 1/3 ppd.

10. The participant has evidence or treatment of malignancy (other than localized skin cancer that has been resected) within the previous 5 years.

11. Plans to undergo a major medical intervention such a surgery within 6 months of enrollment or during the study, or had major surgery in last 6 months.

12. Current or past history of chronic pain syndrome other than IBS in the IBS group (pain >6 months at any location).

13. Any other condition that the investigator believes would jeopardize the safety or rights of the subject or would render the subject unable to comply with the study protocol or make use of acquired data non-analyzable.

14. Use of antibiotics or probiotics within 3 months of the study enrollment.

15. Subjects who currently actively practice CBT or have completed a CBT therapy within 2 years prior to enrolling.

16. Must must have a score of ≥175 (at least moderate severity IBS) on the IB-SSS symptom severity scale completed at screening.

17. Subjects must declare an average abdominal pain in the past 1 week of at least 3 on a 0-10 point scale where 0=no pain and 10=worst imaginable pain.

18. Left handed subjects due to importance of laterality in brain imaging analysis

SPECIFIC AIMS

Cognitive Behavioral Therapy (CBT) is the most well researched and most effective treatment for IBS that targets the brain-gut-microbiome (BGM) axis, and preliminary data show that this therapeutic effect is associated with a reduction of brainstem connectivity with other brain networks. Alterations in BGM interactions as outlined in Overview play an important role in the maintenance and exacerbation of IBS symptoms.[1, 2] The increased prevalence of IBS in women, the higher rate of comorbid non-GI pain conditions, as well as increased sensitivity to a variety of internal and external stimuli (multisensory sensitivity) in women

with IBS suggest the presence of important sex differences in some of these BGM mechanisms. Research performed by the UCLA SCOR during the 3 previous funding cycles (see publication list in Overview) has established an increased responsiveness of the CRF-Locus Coeruleus (LC) system in female IBS subjects, suggesting that this noradrenergic brainstem system plays an important role in IBS pathophysiology.[3-5] In addition, our earlier research has begun to identify clinical, functional and cortical brain mechanisms that may underlie these sex effects. Based on these preliminary data, we will use CBT as a probe to study the relationship between specific disease-related alterations of the brain, the gut microbiome, and symptomatic outcome, and identify the role of sex differences in these relationships. The **overall goal of this project** is to identify sex differences in CBT responders, in the response of the emotional arousal and salience brain networks and in gut microbiome parameters, and to examine clinical characteristics that are associated with these responses. This **study will be the first** of its kind to directly study sex differences in BGM pathways in relationship to a clinically effective IBS treatment, and will therefore both serve to expand and validate the cross-sectional findings from Project 2 as well as provide unique data regarding mechanism of action for a centrally targeted IBS treatment. The proposal aims to test the following **general hypotheses:** 1) Positive clinical response to an IBS-targeted CBT is associated with alterations in specific brain and brainstem regions related to sensory processing and perception; 2) CBT-induced brain changes are associated with alterations in neuroactive gut microbial metabolites suggesting a possible causal relationship and 3) There are sex differences in these BGM interactions that can lead to differential treatment outcomes between female and male patients. We will test these hypotheses in three specific Aims in **male and female IBS subjects**.

Aim A: Identify sex differences in the impact of CBT on the connectivity between brainstem nuclei and brain networks, and between these networks at rest and after provocation.
Hypothesis (H)A1: CBT will result in a *reduction* of various measures of functional and anatomical connectivity of brainstem nuclei with cortical brain networks, and between salience, emotional arousal and sensorimotor networks compared to the usual care condition, and this effect will be most pronounced in CBT responders. **HA2**: Female IBS patients will show greater alterations in the functional and anatomical connectivity of arousal and salience networks at baseline compared to men, and female responders will show the largest changes in these networks after treatment. **HA3**. Changes in multisensory sensitivity and declines in non-GI symptoms will be associated with changes in brain connectivity.

Aim B: Identify sex differences in the impact of CBT on gut microbiome parameters
HB1: CBT in responders will result in a shift of relative abundance towards Bacteroides taxa, and a reduction of the plasma KYN/TRP ratio. **HB2**: Female IBS patients will show greater alterations in the microbiome at baseline compared to men, and female responders will show the largest changes in gut microbiome after treatment. **HB3**: Changes in microbiome parameters will be correlated with changes in brain connectivity.

Aim C: Identify clinical, brain and gut microbiome predictors of CBT outcomes
HC1: Overall IBS patients with a greater degree of alterations in brain network connectivity, including those with higher multisensory sensitivity, somatic co-morbidity and alterations in the corticopontine networks during pain expectation will be less likely to respond to CBT. **HC2**: Exploratory analysis of the broad range of pre-treatment variables will be able to identify specific subgroups more or less likely to show clinical benefit from CBT and any sex differences in these subgroups.

Impact: No prior studies have directly studied sex differences or the range of BGM variables in relationship to CBT treatment for IBS, and we therefore anticipate the results of the proposed studies to provide unique and important new data regarding pathophysiological mechanisms, and personalized clinical response in IBS. The longitudinal study of clinical measures with brain and gut microbiome- based biomarkers in this application should greatly enhance our understanding of sex differences in IBS and help identify biologically based subtypes that lead to more effective, personalized interventions.

RESEARCH STRATEGY

(3A) SIGNIFICANCE

Scientific Premise

Sex as a Biological Variable (SABV) in clinical and biological responses to Cognitive Behavioral Therapy (CBT). Although behavioral interventions and specifically CBT has been found to be among the most effective interventions for improvement of IBS symptoms, there has been limited attempts to test how BGM alterations in IBS are impacted by CBT. Even less is known regarding: if there are sex differences in these effects, if biologically based subgroups of patients exist that show most favorable responses, and if these outcomes can be predicted from baseline variables. As stated in the Overview we have developed a BGM model of IBS based on a large number of previous studies (see publication list in Overview) which allows clear hypotheses regarding how the brain and microbiome may interact in IBS, and how SABV affects these interactions. The UCLA SCORE proposal aims to test these hypotheses in 3 interdependent and synergistic Projects, as indicated in **Fig. 1.** The current Project 3 aims to test the model using a clinically effective intervention. We have tested the effect of CBT in a preliminary study which showed that that CBT responders were predicted by greater relative abundance of Clostridiales taxa at baseline, had a greater shift towards Bacterioides post treatment, and showed a significant reduction in connectivity between brainstem and relevant brain networks, and between emotional arousal, salience and sensorimotor networks (**Fig. 8**).[6, 7] However this CBT trial was not designed to determine sex differences, as an insufficient number of male IBS patients were included, and brain imaging was not optimized for identification of specific brainstem nuclei. Based on these preliminary data, **we hypothesize that sex-related differences in BGM interactions will impact treatment response and that examining the response to a selective, brain targeted intervention (e.g. CBT) is a powerful tool to validate which aspects of BGM interactions are most important**.

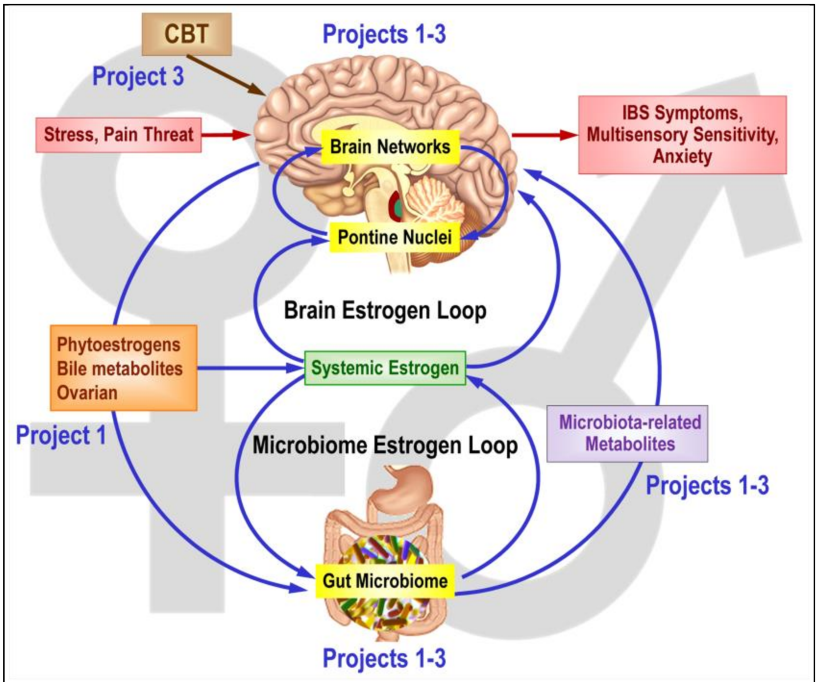


Fig. 1. BGM model for IBS with SABV. Model shows bidirectional interactions between brain, gut, gut microbiome and estrogen. At the brain level, bidirectional interactions exist between cortical networks and pontine nuclei. Shown are aspects of the model addressed by the 3 SCORE Projects.

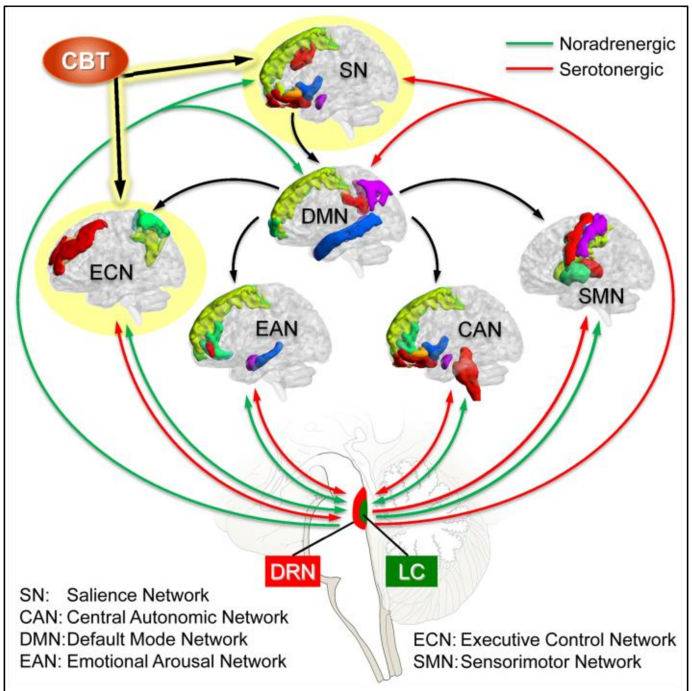


Fig. 2. Bidirectional connections between dorsal raphe nucleus (DRN) and locus coeruleus (LC) and various brain networks. Noradrenergic projections (shown in green are excitatory, serotonergic (shown in red) are inhibitory. CBT is hypothesized to normalize altered activity and connectivity of SN, ECN and EAN.

Importance of brainstem nuclei in BGM interactions. An extensive literature suggests a prominent role of the LC (and its central ascending *noradrenergic* projections) and of the DRN (and its ascending *serotonergic* projections) in the pathophysiology of increased emotional arousal and stress responsiveness in IBS[8], as well as several psychiatric disorders characterized by increased emotional arousal which have a greater prevalence in women (**Fig. 2**).[9-11]

Modulation of brainstem nuclei by descending projections from emotional arousal and salience

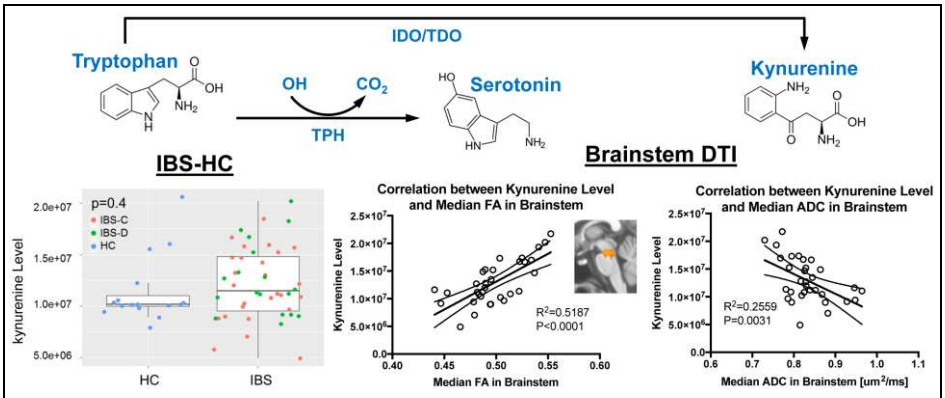


Fig. 3: Association between gut microbial plasma metabolites (shown for the tryptophan metabolite KYN and DTI measurements of dorsal brainstem in IBS.

networks. The LC receives strong input from regions of the salience and emotional arousal networks.[12] Support for a crucial involvement of these brainstem nuclei in disorders of chronically increased emotional arousal comes from studies in postmortem tissues for the pontine LC,[13] and the DRN,[14, 15] showing degenerative changes and reduction in cell numbers in patients with brain disorders characterized by chronic overstimulation of the central noradrenergic system (suicidality, depression, chronic stress). As this cell loss in the LC can be associated with *increased* activity of tyrosine hydroxylase, the rate limiting enzyme in norepinephrine synthesis such degenerative changes are not necessarily associated. This is consistent with our own preliminary findings of neuroplastic changes in the dorsal brainstem in IBS subjects (**Fig. 3**).

Modulation of brain networks by ascending projections from brainstem nuclei. The LC sends largely excitatory, noradrenergic projections to the brain, including regions of the salience, emotional arousal and sensorimotor networks, as well as to many brainstem nuclei, including the DRN.[16] Besides the close reciprocal interactions with the LC, the DRN sends extensive projections to many of the same brain areas including the amygdala, thalamus, and most of the cortex. It integrates a vast amount of information from the brainstem and influences virtually all regions of the brain. However, despite the known relevance in the regulation of cortical and limbic brain networks, and their importance in BGM communication, brainstem regions and their influence on the connectivity and function of brain networks, and their possible role in the therapeutic effects of CBT have not been studied in detail in human brain imaging studies.

Sex differences in pontine emotional arousal systems. An extensive literature suggests a sex-related, prominent role of the LC,[11, 17] and central ascending noradrenergic and serotonergic system in the pathophysiology of increased stress responsiveness in clinical syndromes associated with emotional hyperarousal, including PTSD and IBS[8], all of which are more common in women.[3, 11] Estradiol increases NE synthetic enzymes and reduces NE degradation, suggesting that estradiol mediated increase in NE signaling to the brain increases arousal and maybe contribute to the greater prevalence of disorders characterized by hyperarousal.

***The role of the gut microbiome in modulating brain networks*.** Based on our preliminary results (**Fig. 3**), we hypothesize that nuclei in the dorsal pons can be influenced by gut microbial metabolites acting on spinal and vagal afferents in the gut, or reaching the pons via the systemic circulation. Preclinical studies support the importance of vagal afferents in transmitting microbial signals from the gut to limbic brain networks, and given the close connections between the vagal nucleus tractus solitaries with LC and DRN,[18] these nuclei may serve as a relay in distributing microbial information to the brain.

In summary, based on our preliminary results, **we hypothesize** that brainstem nuclei in IBS patients are chronically stimulated by descending projections from regions of the salience and emotional arousal networks (anterior insula [aINS] and amygdala), that LC and DRN show exaggerated responses to a pain threat task, and that a reduction of these stimulatory inputs associated with CBT may be an important mechanism underlying CBT effectiveness for IBS (see Aim 3). In addition to descending excitatory influences from the brain, gut microbial metabolites and female sex hormones provide additional modulatory input to these nuclei. Recognizing the known complexity of connections of brainstem nuclei amongst each other, with the brain and with the spinal cord (descending pain modulation), the focus of the current proposal is on LCC and DRN, as a

critical source of ascending modulatory influences on cortical brain networks activity and connectivity.

Importance of problem.
The great majority of published studies on BGM interactions have been performed in rodent models, but the translational relevance of alterations in the gut microbiome and the resulting altered signaling to the brain for human brain gut disorders such as IBS remain to be determined. Furthermore, sex differences in BGM interactions in humans have not been reported to date, even though an extensive preclinical literature supports an important role. Given the potential importance of BGM interactions in determining sex differences in IBS presentation and treatment, a better understanding of the mechanisms addressed in this proposal, in particular those predicting treatment responses and which underlie symptom improvement by CBT, hold promise for development of new treatment targets and modifications of known treatments to better address sex differences.

Current barriers to progress.
No information on sex differences in non-pharmacological IBS treatment: A growing number of studies demonstrate the effectiveness of non-pharmacological interventions and especially CBT for the treatment of IBS,[19-22] both compared to control conditions and pharmacological treatments. This includes two recent controlled trials examining the home-based CBT intervention proposed for this project.[23, 24] **However, only small numbers or no male patients have been included in these trials and therefore it is not known how SABV and gut microbial metabolites may impact outcomes.**

Lack of biological models for how and for whom CBT treatments work: As a centrally targeted treatment, CBT is hypothesized to positively impact the brain networks related to salience assessment (reduction of biased predictions), heightened emotional arousal and negative affect associated with IBS.[25, 26] Although clinical responses in IBS suggest this may be the case, little research of these mechanisms in the context of CBT treatment has been performed. Our preliminary studies, examining both brain and microbiome data from a subsample of the recently completed IBSOS CBT study[23] provides preliminary evidence that CBT responders show a significant reduction in connectivity between brainstem and relevant brain networks, and between emotional arousal, salience and sensorimotor networks (**Fig. 8**) and that some of these connectivity changes are associated with microbial taxa that predict treatment outcomes (**Fig 9**). These findings are consistent with our hypothesis about involvement of LC and or DRN in CBT response. In addition, responders showed a greater relative abundance of Clostridiales taxa at baseline (**Fig. 11**), and a greater shift towards Bacteroides post treatment (**Fig. 10**). Sex differences were not examined due to a too small male group. Based on these preliminary results showing significant effects of CBT at different levels of the BGM axis associated with symptom improvement, **the proposed study will be the first to comprehensively study these brain and gut microbiome variables in both men and women before and after CBT.**

Limited methodologies to study the role of brainstem nuclei in IBS. As detailed in the **Overview** and **Project 2** of this proposal, previous studies using brain imaging techniques in IBS: 1) Did not focus on brainstem; 2) Did not have the spatial resolution to differentiate between brainstem nuclei of interest, e.g. LCC and DRN, or to identify effects of gut microbial metabolites on their structure and function. In addition, the great majority of imaging studies were cross sectional, only showing associations without demonstrating causality. The proposed combination of novel imaging and advanced bioinformatics methodologies and a clinical trial design with an effective intervention have the potential to overcome these previous barriers to understanding alterations in the human BGM axis. The current Project adds the benefit of a longitudinal design with a centrally targeted intervention which effectively reduces IBS symptoms and can alter BGM interactions to the advanced biological markers proposed in the Project 2 and will provide mechanistic information on IBS recovery.

Impact. The identification of biological mechanisms underlying CBT responses, and the identification of patient subgroups (including those based on SABV) with differential responsiveness to CBT will lead to personalized interventions, including pharmacological and non-pharmacological brain- and gut-microbiome-targeted therapies.

(3B) INNOVATION

- **Using CBT as a probe, identifying evidence for sex-specific causal relationships between nervous system alterations, gut microbial metabolites and IBS symptoms.** We will examine a validated CBT intervention which has recently been shown by one of our co investigators to reduce IBS symptoms[23] and which our preliminary results have shown to normalized aberrant brain connectivity as well as gut microbial composition and function.[6, 7] In the proposed study we will build on these observations in two important ways: 1) We will examine an equal number of female and male patients with CBT and a control condition to determine if SABV influences BGM mechanisms associated with treatment response. 2) We will utilize more brain imaging methodologies with greater spatial resolution, novel bioinformatics approaches and microbiome analysis methods that enable more detailed evaluation of involved brain stem mechanisms and microbiome parameters.
- **Use of advanced techniques and analytical approaches to integrate measurements at different level of the BGM axis to improve identification of differences between responders and non-responders to CBT.** As detailed in **Project 2** and the Methods section below, we will include high resolution MRI scanning of cortical, subcortical and brainstem regions to provide a more comprehensive assessment of changes in relevant brain networks targeted by CBT, and to evaluate the relationship of these networks to gut microbial parameters. Our preliminary data (**Fig. 3**) is consistent with neuroplastic changes in the brainstem/midbrain areas in IBS which are correlated with plasma levels of the TRP metabolite kynurenin (KYN). This will be the first study involving an IBS treatment linking gut microbial measurements with alterations in brainstem nuclei and their ascending noradrenergic and serotonergic projections that may underlie IBS related alterations in the modulation of brain networks.

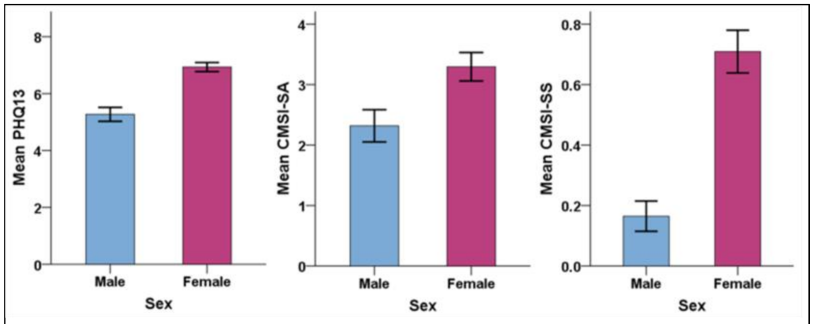


Fig. 4. Sex differences in scales of co-morbid symptoms and multi-sensory sensitivity.

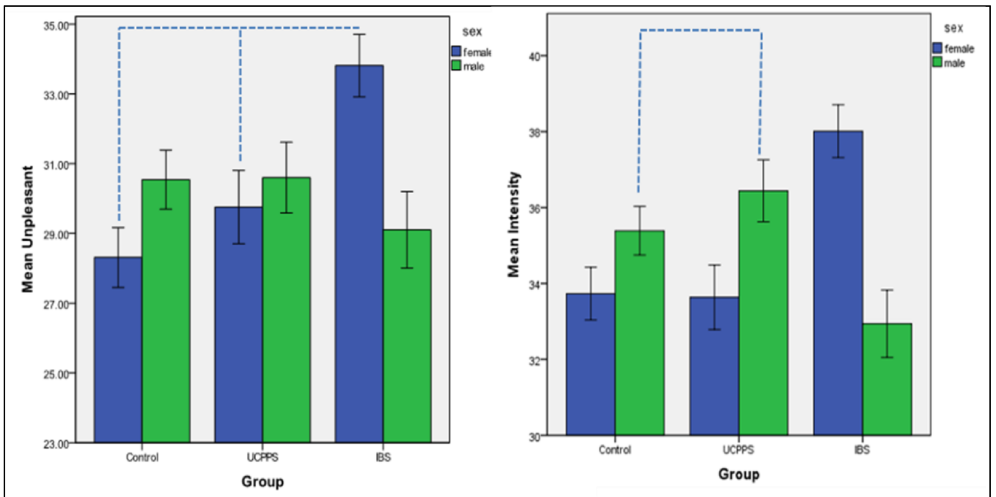


Fig. 5. Increased unpleasantness and intensity ratings of unpleasant mixed frequency sounds in female IBS compared to males, HC, and chronic urological pelvic pain (UCPPS)

- **Evaluation of CBT impact on mechanisms of multisensory sensitivity and co-morbidity.** IBS is now recognized as one of 10 chronic overlapping pain conditions (COPCs) which also include fibromyalgia, migraine headache, vulvodynia, and chronic pelvic pain conditions. These disorders commonly co-occur with each other, as well as with psychiatric comorbidities characterized by emotional hyperarousal (in particular anxiety, depression and PTSD), and are thought to share some pathophysiological characteristics such as multisensory sensitivity and deficits in central pain modulation.[27] Of particular importance for this Project, most COPCs also occur more frequently in women than men, and are often resistant to pharmacological treatments.[28, 29] Even after accounting for reporting bias, IBS prevalence is significantly higher in women[30] and women with IBS show increased rates of wide-spread symptoms and visceral hypersensitivity.[31]

Figure 4 shows data from a large sample of men and women with ROME+ IBS seen in our Center. Although

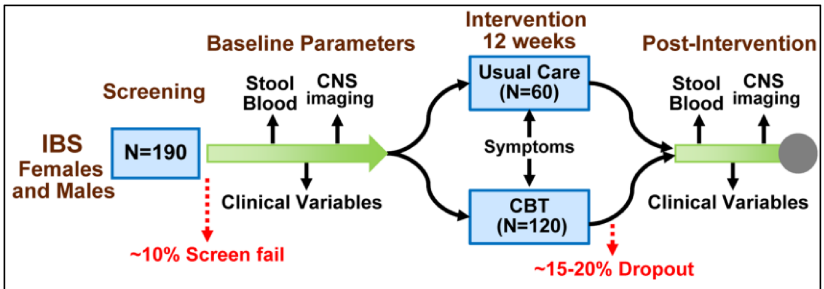


Fig. 6. Study Design. 190 premenopausal female and male IBS subjects will phenotyped at baseline, and then randomized into the CBT (n=120) or Usual Care (n=60) groups. Phenotyping studies will be repeated following completion of interventions at 10 weeks.

their overall IBS symptom severity was similar, women with IBS showed significantly higher co-morbid somatic symptoms on the Patient Health Questionnaire somatic index (PHQ13) as well as higher scores on the Chronic Multisystem Symptom Index (CMSI) scales for sensory sensitivity and somatic awareness. We have also shown that women with IBS show a greatly enhanced sensitivity to unpleasant sounds generated by mixed frequencies of tones compared to HCs, while men with IBS are similar or less sensitive than HCs (**Fig. 5**). EEG and fMRI studies also report enhanced brain responses to auditory stimuli[32-35] suggesting altered sensory processing in IBS may not be restricted to visceral sensation. Similar findings of increased multisensory sensitivity and higher rates of comorbidity in women compared to men have also been reported for other chronic pain conditions such as urological chronic pelvic pain syndrome (UCPPS)[36] but to date there has been very limited research of either the biological underpinnings of these differences, or their impact on symptomatic treatment. We have recently shown in the NIDDK-funded MAPP study that the presence of more widespread symptoms in UCPPS patients is associated with altered brain connectivity involving the sensorimotor and salience networks, a finding consistent with our hypothesis.[37] However, this study did not address potential sex differences in these responses, nor did it take a role for monoaminergic ascending modulation into account. Based on the general model of the current proposal (**Figs. 1, 2**) we hypothesize that an important component of sex differences seen hypersensitivity and co-morbidity in premenstrual female IBS results from estrogen and/or estrogen metabolite modulation of the monoaminergic ascending systems originating from pontine LC and DRN. Preclinical and some clinical evidence suggests a stimulatory effect of estrogen on both systems. As women have a reduced enzymatic capacity to generate serotonin in the brain, the stimulatory effect of estrogen on the noradrenergic arousal system, and the resultant cortical arousal is expected to be more pronounced. **In summary, the proposed project is highly significant as it will directly test mechanistic hypotheses related to sex differences in multisensory sensitivity and comorbidity in IBS as well as the impact of these differences on response to an effective IBS treatment.**

(3C) APPROACH

Overview and Preliminary Data.

This Project will apply advanced neuroimaging acquisition and advanced bioinformatics techniques to test the general hypothesis that the clinical response to CBT is accompanied by functional alterations in cortical and brainstem networks **(Aim A)** as well as serum and gut microbial parameters (**Aim B**). We also hypothesize that some aspects of these alterations will be sex-specific, and that some BGM interactions at baseline will be predictive of positive response **(Aim C)**. The studies, summarized in **Fig. 6**, will collect advanced neuroimaging data from brain and brainstem, and microbiome data from stool and blood before and after CBT treatment and a 'usual care' control, linking treatment response with these biomarkers and non-GI markers including multisensory sensitivity, pain co-morbidity and symptom-based anxiety. SABV will be assessed at every level of the analysis. The proposed studies aim to test the following hypotheses**:**

<u>Aim A:</u> Identify sex differences in the impact of CBT on specific brain networks at rest and after provocation.

In order to go beyond the identification of cross sectional associations between different components of the BGM axis and behavioral data and look at mechanisms of treatment response, we will examine brain responses to an exclusively centrally targeted intervention, CBT, which we have recently demonstrated to effectively reduce IBS symptoms.[23]

Effect of CBT on brainstem and brain network connectivity. **Fig. 7, right panel** shows connectograms visualizing functional connectivity changes in response to CBT in responders (left) and non-responders (right). Blue lines show significant *reductions* in connectivity between different brain networks (color coded). The great majority of these changes were not seen in non-responders (right panel). **Most relevant to this proposal**, there was a *reduction* in connectivity between brainstem (bottom of circle in black) and sensorimotor network and bilateral default mode network (d= -0.56 to -0.71), consistent with either a reduction in the influence of ascending monoaminergic modulatory systems originating in the brainstem region on these cortical networks, or a reduction in the *top down* modulation of the brainstem nuclei. In addition, a reduction of connectivity between sensorimotor, default mode, emotion regulation and salience networks was observed. The widespread nature of these changes suggest a CBT induced change in modulatory influences originating from the brainstem affecting widespread brain regions. **Fig. 7**, left panel shows significant differences in **neuroplastic changes in the dorsal brainstem** region between responders and non-responders to CBT, revealed by DTI imaging of the brainstem. Responders had a significant *increase* in apparent diffusion coefficient (ADC) within the dorsal brainstem relative to baseline (P=0.0015), while non-responders had a significant *decrease* in ADC (P<0.0001). The difference between responders and non-responders was also significant (P<0.0001).

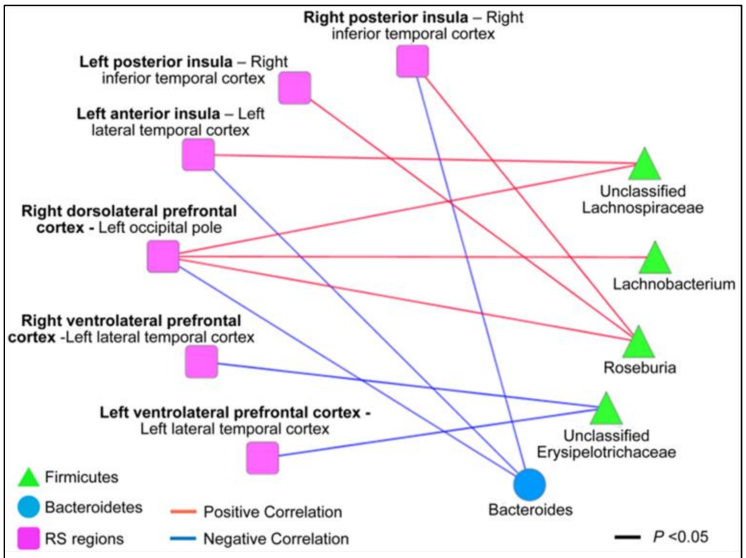


Fig. 8. Partial correlation analysis between microbial genera which predict CBT outcomes RS connectivity between brain networks.

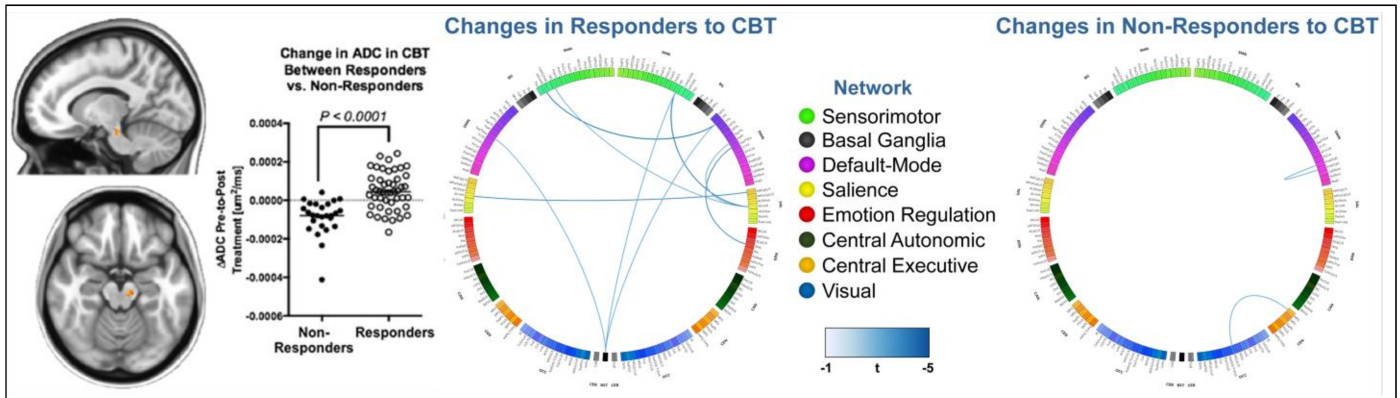


Fig. 7. Structural (left panel) and connectivity changes (right two circles) of brainstem region containing LC and DRN in CBT clinical responders.

If patients were stratified based on a positive or negative change in ADC within the dorsal brainstem there was a 72% sensitivity and 86% specificity of detecting CBT responders (ROC analysis, AUC=0.86, P<0.0001). **In the current proposal** we will expand the sensitivity of our brain stem imaging approach in several ways including using a 7T MRI scanner, and locus coeruleus specific imaging technique, and by using a provocative pain threat task to activate the arousal systems.

CBT induced changes in gut microbial abundance and correlation between brain connectivity changes and gut microbes. We performed a partial correlation analysis between microbial genera which were found to predict CBT outcomes (Firmicutes/Clostridiales) and RS connectivity in CBT controlling for sex and IBS-type, which revealed several positive and negative associations with connectivity of the insular (top three pink squares) and regions of prefrontal cortex (lower three pink squares) (**Fig. 8**). For example, the change in the abundance of *Bacteroides* (negative predictor of outcome) was negatively associated with the decrease in RS connectivity including left transverse temporal sulcus to left short insular gyrus (r=-.31, p=.03). On the other

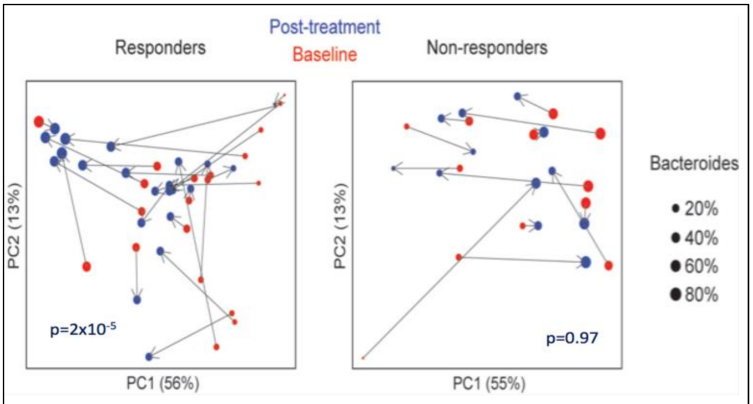


Fig. 9. Differences in CBT induced shift in relative abundances of the genus *Bacteroides* between responders (left panel; consistent leftward shift) and non-responders (inconsistent shifts).

hand, alterations in the abundance of *Roseburia*, a genus of the Clostridiales order (a positive predictor of outcome) was positively associated with several insular cortical connections (r=.3, p range=.01-.04). However, in these preliminary studies, FDR corrected *P* values did not reach significance due to the insufficient sample size. **In summary**, these preliminary results suggest that IBS symptom improvement is associated with functional and structural connectivity changes, some of which involving brainstem regions, and that these brain changes are correlated with changes in gut microbial taxa.

Activation of arousal circuits from experimentally induced pain threat. We have validated procedures for eliciting expectation of a painful abdominal stimulus that results in a robust activation of emotional arousal circuitry and a significantly greater response in IBS patients.[38-40] These procedures contrast 'anticipation' periods in which an electrical stimulus may be applied to the lower left abdomen over the sigmoid colon with 'safe' periods in which no stimulus is expected. We found large auditory evoked eyeblink responses during anticipation compared to safe conditions, reflecting the engagement of upregulated arousal networks and greater arousal responses in IBS patients.[38] Using a similar procedure adapted for the MRI environment we have shown that anticipation of abdominal pain is associated with engagement of several cortical and limbic brain regions with significantly greater responses in the dorsal brainstem region and hypothalamus in female IBS patients compared to female HCs.[39] In a follow up study we have shown that IBS subjects showed greater activations in affective (amygdala, INS), sensory (thalamus), attentional (medial frontal gyrus) and self-referential (precuneus) regions during this anticipation procedure. In addition, these greater brain responses were primarily observed between female IBS subjects and female HCs.[40]

Relevance for current project: The proposed studies examine hypotheses regarding specific IBS alterations in brain stem and cortical networks involved in arousal, salience assessment and attention, and sex differences in these responses. Although we have shown differences in these networks in the resting state, the use of a targeted and well validated provocation task should lead to more robust findings and be especially important when examining associations of brain network responses with microbiome responses and individual differences in response to a CBT intervention. We will therefore include a version of the visceral pain anticipation procedure in each of the MRI sessions along with resting state functional and structural protocols.

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Aim B: Identify CBT-induced changes in gut microbiota. As shown in **Fig. 9, left,** CBT responders showed a consistent shift towards greater abundance of the genus *Bacteroides* (shown as uniform leftward arrows in left panel), while these shifts were inconsistent in non-responders (right panel). This shift in relative *Bacteroides* abundance was accompanied by the induction of 5 bacterial genera, most notably *Bacteroides* (2.5 fold, $q=4x10^{-5}$; results not shown). **In summary**, our preliminary data demonstrates that the subjective CBT response is associated with both changes in structural and functional connectivity of specific brain and brainstem regions (**Fig. 7, above**), and with an increase of taxa in the phylum Bacteroidetes, including several taxa, which have previously been shown to be involved in host serotonin synthesis.[41] Although men and women were enrolled in this pilot study the focus was not on sex differences or recruiting equal numbers of men and women and sex differences were not evaluated. The proposed study aims to confirm the findings and to determine possible sex differences in these relationships.

Aim C: Identify clinical, brain and gut microbiome predictors of CBT outcomes. A further goal of this Project is to examine baseline BGM parameters as potential predictors of CBT response. Our analysis of the microbiome from a subset of patients receiving CBT in the IBSOS CBT trial[24] showed CBT responders had higher relative abundance of Clostridiales, and higher fecal serotonin levels compared to non-responders ($p<.01$,**Fig. 10, left two images**). In the same sample a baseline microbiome classifier predicting responder status including 11 genera, and 3 enriched Clostridiales was identified ($p<.05$; **Fig. 10, right**). In the same study [24], we have recently identified a clinical phenotype among IBS patients encompassing trait anxiety, anxiety sensitivity, intense worry, and somatization that is negatively related to CBT outcomes. Patient clusters scoring low, medium and high on these measures showed significantly different responses to CBT ($\chi 2$ (df = 2, n = 144)= 6.78, p = .034) with the low cluster at 70% positive response and the high cluster at 40%. We hypothesize that these symptom-based clusters based on clinical response will be correlated with gut microbial

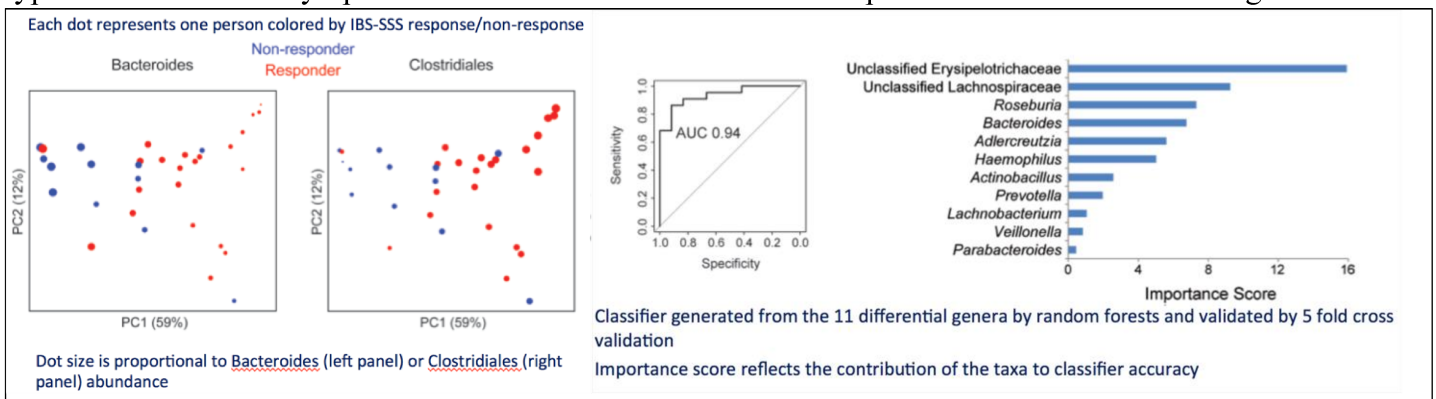


Fig. 10. Left side: Increased relative abundance of Clostridiales (right panel) at baseline was predictive of CBT clinical responders, while Bacteroides abundance was predictive of non-responders (left panel). Right side: Prediction of responder status by classifier (mostly Clostridiales) generated from 11 genera.

based subgroups. We have also shown in a longitudinal prospective study of patients with Urological Chronic Pelvic Pain (UCPPS) that symptom outcome over three months is predicted by the functional connectivity between several brain networks also shown to be altered in IBS involved in attentional modulation and selection of sensory information by attention.[37] Again these studies did not have the sample to test for sex differences.

General Methods.

Human subjects. A total of 90 men and 90 women with IBS will be enrolled in the protocols for this Project in years 1-4. All participants will be evaluated on the study measures at baseline and three months later. Two thirds of the participants will be randomly assigned to receive the CBT intervention and one-third will be designated usual care and only receive the assessments. Based on our prior studies we estimate about 15% loss of data from subject withdrawal resulting in sample sizes of 100 CBT (half female) and 50 usual care (half female) for a per-protocol analysis Similar to our previous studies, the subject population will be composed all bowel habit subtypes with ages of 18-55 years. Details of enrollment criteria and exclusion factors, including medications, and questionnaires to assess IBS symptom severity can be found in the Protection of Human Subjects section.

Minimal contact CBT: This intervention (referred to as CBT throughout this proposal) will use the CBT protocol developed by co-investigator Dr. Jeffrey Lackner which has shown excellent efficacy (61% symptom responder) compared to an education control (44% responder) in a recent large randomized multisite trial.[24] The effects of CBT on brainstem connectivity (**Fig. 7**), and the role of the gut microbiome in the moderation and mediation of the effects in responders are shown in **Figs. 9,10**, above. Minimal contact-CBT is primarily a home based intervention that has shown the same efficacy as a more standard 12 session in-office CBT in two controlled trials.[24] It involves only **4 in-person 60 min sessions** over 10 weeks and several phone contacts. Two therapists who have been trained by Dr. Lackner will perform the CBT and will have regular teleconferences or in-person meetings with Drs. Lackner and Naliboff to ensure integrity of the treatment protocol. A **responder** will be defined as a subject with IBS-SS score change of ≥50 from baseline to end of treatment and corresponds to a clinically meaningful change in symptoms[42]. Subjects will also record daily

symptoms (pain on a 0-10 NRS[43] bloating 0-10 NRS, and stool frequency and form [BSFS][44]) on an electronic diary for 1 week prior to starting the CBT and for one week during the 5th and 10th weeks of CBT. Participants assigned to the Usual Care condition will have the same questionnaire, neuroimaging, biological and symptom diary assessments as those in CBT but will not have any specific intervention from the project.

Aim Specific Methods:

Aim A: Identify sex differences in the impact of CBT on the connectivity between brainstem nuclei and brain networks and between these networks at rest and after provocation.

Neuroimaging Data Acquisition and Post-Processing. Before and after treatment, all participants will undergo a 90 minute structural, resting state, evoked-task, DTI and brain stem MRI scanning sessions will acquire data throughout the brain and brainstem, including the LCC and DRN. As a part of Project 2, **Brain and Brainstem Microstructure will be quantified using Diffusion Spectrum Imaging (DSI) (**514 direction, multishell DSI sequence, 2mm isotropic resolution throughout the entire brain). Additionally, **locus coeruleus-specific imaging will be performed "Neuromelanin-sensitive-weighted" MRI and T1 Mapping.** The function and morphology of the LC in IBS and HC will be compared by exploiting the T1 shorting properties of neuromelanin and its documented relationship to function of the LC.[25] All subjects will be imaged on a Siemens 3 Tesla Prisma scanner housed at the UCLA Ahmanson-Lovelace Brain Mapping Center (ALBMC). Details regarding MRI acquisition and post-processing and analysis can be found in the Neuroimaging section of the Data Processing and Analysis Core (DPAC) and in Project 2.

Pain Threat Protocol (Fig. 11). This protocol will follow procedures successfully used in our previous studies.[39, 40] Before entering the scanner, participants will undergo a pain threshold assessment. Delivery of transcutaneous electrical stimulation to the abdomen will utilize a Digitimer constant-current stimulator (model DS7A; Digitimer) and two electrode stimulation pads placed 6 cm apart over the subject's lower left abdomen in the region overlaying the sigmoid colon. Each stimulation to the abdomen will consist of a pulse train lasting 750 ms with a 2 ms pulse width and a frequency of 37 Hz. For each subject, a moderately intense but not intolerable pain threshold (in milliamperes) is determined using a method of limits procedure beginning with a current intensity of 1.0 mA, which is increased in 0.5 mA steps until the subject reports the stimulus as "aversive but tolerable." This level is then used during the pain threat scanning.

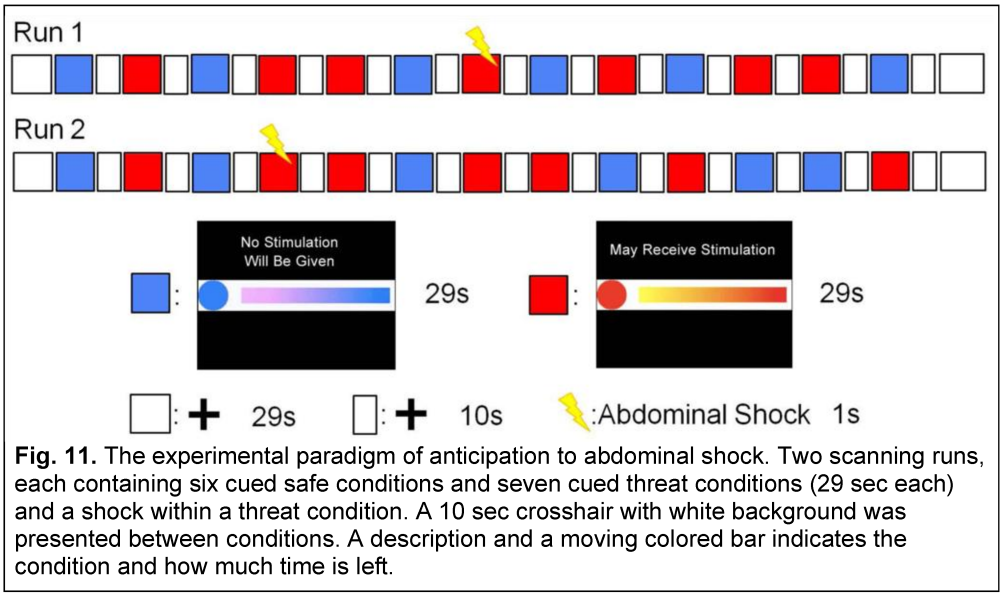


Fig. 11. The experimental paradigm of anticipation to abdominal shock. Two scanning runs, each containing six cued safe conditions and seven cued threat conditions (29 sec each) and a shock within a threat condition. A 10 sec crosshair with white background was presented between conditions. A description and a moving colored bar indicates the condition and how much time is left.

Details of the Pain Threat scan procedure are described in the DPAC. Briefly participants will have video goggles placed to view the paradigm. They will be instructed that during a blue cue there will be no chance of an abdominal stimulation (safe condition) but during the orange cue there will be a chance of a "safe but painful" abdominal "shock". The color of the cues will be counterbalanced between subjects. The subject will then undergo fMRI scanning during two runs of 6 safe and 7 threat trials of 29 seconds each in randomized

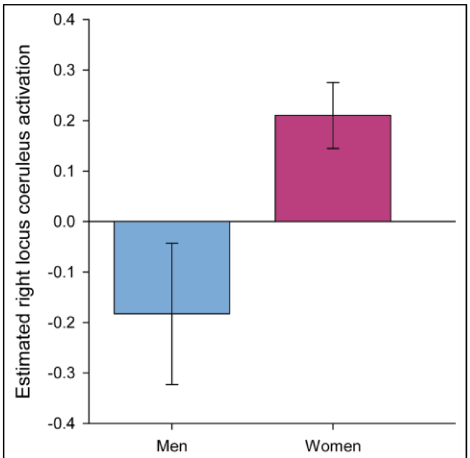


Fig. 12. Locus coeruleus activation by threat of an abdominal shock is initially greater in female patients than in male patients with IBS (corrected p=.02).

order with a neutral intervening crosshair condition (10 sec) between trials (see **Fig. 11**). During each trial a moving bar will show the amount of time left in the trial. Two actual shocks will be given one in the first half of the trial and on in the second half. In preliminary studies, locus coeruleus activity in response to the threat (vs safe) condition was compared between male and female patients with IBS (n=22 and 62, respectively), controlling for mean framewise displacement (a measure of motion severity). Female patients showed greater activation of the right locus coeruleus compared to that in male patients in the first half of the study (T=2.69, corrected p=.02), but not in the second half of the study (p=.92) (see **Fig. 12**).

Analysis:

Aim B: Identify CBT-induced changes in gut microbiota.

Intestinal Microbiome Analyses: These studies will be conducted in the UCLA CURE Microbiome Core. DNA extraction from stool samples and sequencing of the V4 region of 16S rRNA on the Illumina HiSeq 2500 platform will be performed according to a published protocol.[45] Extracted DNA will undergo shotgun metagenomics sequencing on an Illumina NovaSeq 6000 to a depth of 20 million sequences per sample. Additional details are provided in the Data Processing and Analysis Core.

Plasma and fecal metabolomics: Blood and stool samples will be collected from patients at the time of each MRI visit. Blood samples will be centrifuged to obtain platelet-depleted plasma. Plasma and fecal samples will be frozen until all studies are completed. Aliquots of plasma will be analyzed in a single batch on their global untargeted metabolomics and bioinformatics platform (Metabolon, Durham, NC).[46] Datasets provided by Metabolon will be analyzed for several hundred named compounds including amino acids and their derivatives, products of primary metabolism, bile acids, neurotransmitters (e.g. 5-HT, TRP, kynurenine, dopamine, gamma-Aminobutyric acid [GABA]), endocannabinoids, steroids, and fatty acids. Stool will undergo targeted analysis with panels for bile acids, estrogen metabolites and short chain fatty acids. **Additional details regarding fecal and plasma metabolomics can be found in the Microbiome Sub-Core.**

Self-report Clinical Measures: In addition to the IBS-SS, validated clinical measures including those for IBS symptom anxiety, pain and affective co-morbidity, multisensory sensitivity and IBS related cognitions (e.g. catastrophizing) will be given at baseline and post-treatment. See for Human Studies section for details**.**
IBS Symptoms: Primary outcome will be IBS-SS. Additional measures of IBS symptoms will be the one week electronic symptom diary (pain on a 0-10 NRS[43] bloating 0-10 NRS, and stool frequency and form [BSFS][44]) and the Visceral Sensitivity Index (VSI) measuring visceral specific anxiety. **Multisystem sensitivity and pain co-morbidity:** CMSI scales of Somatic Awareness and Sensory Sensitivity,[47] the Sensory Hypersensitivity Scale (SHS),[48] and the recently developed Chronic Overlapping Pain Conditions Screener (COPCs) which includes symptom diagnosis for 10 common pain conditions and a body map of pain sites based on the CMSI.[49]
General psychological functioning and QOL: Supportive questionnaires will include cognitive and affective measures related to chronic pain experience including the Pain Catastrophizing Scale, (PCS),[50] Hospital Anxiety and Depression (HAD) scale,[51] and the Early Traumatic Inventory-Self Report (ETI-SR).[52]

General Analytical Approach

A combination of univariate, multivariate, will be applied to test the hypotheses and aims. Analyses will focus on core regions and functioning of the emotional arousal, central executive, sensorimotor processing, default mode (DMN), and salience networks. After defining regions of interest (ROIs) for cortical and subcortical brain, and brainstem (including the DRN and LCC) with the Human Connectome Project Multimodal Parcellation atlas v. 1[53] (180 cortical regions), Harvard Oxford subcortical atlas[54, 55] (7 subcortical structures) and HCP-based tractographic atlases of the brainstem[56, 57], each will be characterized by their 1) macro (volume, cortical thickness, mean curvature, surface area)- and 2) micro-(FA, MD, TD) structure, and 3) functional and anatomical architecture and connectivity (network metrics, resting state, time series correlations,

number of fibers connecting ROIs)**. Hypothesized group differences in these neurometrics will be tested using contrast analysis within the framework of general linear models (GLM) and all analyses will correct for multiple testing with a false discovery rate of 5%.** Using statistical parametric mapping (SPM) version 12, general linear models be used to analyze the evoked task. Details for microbiome and metabolomics analysis for 16S rRNA sequence shotgun metagenomics and metabolomics are outlined in the Microbiome Sub-Core of the Data Processing and Analysis Core.

Aim-specific Analytical Approaches

Aim A: Identify sex differences in the impact of CBT on the connectivity between brainstem nuclei and brain networks, and between these networks at rest and after provocation.
Full factorial random effects GLMs specifying including sex (male, female), group (CBT, UC), time (BL, Post Treatment) as factors used to test the influence of CBT along with the moderating effects of sex on neurometrics reflecting functional and anatomical connectivity of brainstem nuclei with salience, emotional arousal and sensorimotor networks during the resting state and evoked-task imaging sessions. Additionally, independent components analyses will be used to compute the intrinsic connectivity of the aforementioned brain networks and to index the interactions of these networks with each other, both which will serve as dependent variables in the GLMS. To determine if changes are more pronounced in responders, we will rerun the GLM using a 3 level group factor (CBT responders, CBT nonresponders, UC). GLMs will also be utilized to test whether CBT induced brain changes are associated with reductions in multisensory sensory sensitivity (decreased recruitment of multimodal sensory regions) and declines in non-GI symptoms will be associated with the brain responses described above in brain stem and cortical networks.

AIM B: **Identify sex differences in the impact of CBT on gut microbiome**
Differential abundance of taxa pre-and post-treatment will first be determined by non-parametric tests (Kruskal-Wallis or Mann Whitney U depending on the number of groups in the comparison). Multivariate negative binomial mixed models, implemented in the R package BhGLM, will then be applied to assess effects of sex and allow for correction for covariates that may influence microbial composition with subject treated as a random effect**.** Fisher's exact test will be used to identify differences in the frequency with which strains are present among groups of subjects. Multivariate negative binomial mixed models will be applied to assess for differential strain abundance after adjusting for covariates. Random effects general linear repeated measure models and logistic regression will be used to test the impact of treatment and sex on metabolite concentrations (specifically, estrogen and tryptophan pathway metabolites as well as short-chain fatty acids). The moderating effect of sex will be tested by inclusion as a covariate (i.e., main, interaction effects) in the statistical models. Details on microbiome analyses and computation of outcome variables to be tested are contained in CORE B. A false discovery rate of 5% will be applied for all hypothesis testing.

AIM C: Identify clinical, brain and gut microbiome predictors of CBT outcomes
Logistic regression employing the generalized linear models will be used to test the hypothesis that patients with higher multisensory sensitivity, somatic co-morbidity and alterations in responses of brain stem and cortical networks during pain expectation will be less likely to respond to CBT and will have a positive symptomatic response. The models will allow us to further test the hypothesis that this association will be moderated by sex, with female IBS showing a greater response. To further interrogate the data, a sPLS-discriminant analysis (sPLS-DA) will be used to determine a limited set of features (neurometrics, clinical, and microbiome/metabolome) that best discriminate responders and nonresponder to CBT. This analysis will identify multi-omics associations and patterns that discriminate between responders and nonresponders.

Sample Size and Power. G*Power was used to perform power analyses. Tests of hypothesized group differences are based on contrast analysis on estimated generated from the general linear models. *Preliminary studies suggest CBT induces moderate to large effect size changes in the mean abundance of bacteria and neurometrics*. Sex differences at baseline. The sample of 90 females and 90 males will provide adequate power to detect effect size differences as small as d=.42 for all outcome variables. Impact of CBT. For the comparison

with usual care, 100 CBT and 50 UC provides adequate power to detect an effect sized difference as little as d=.49 if it exist (two-tailed, independent t-tests, α=.05) for response differences the interventions, $t_{crit}(148)=$ ±1.98, noncentrality parameter=2.82). The sample of 100 will provide adequate power (80%) to detect a CBT effect size change as small as d=.45 (two-tailed dependent t-test, α=.05, $t(99)_{crit}=$ ±2.01, noncentrality parameter=2.86). Sex differences in the impact of CBT. A sample of 50 men and 50 women provide adequate power to detect a mean difference in the effect of CBT on outcomes as large d=.50.

Proposed Studies.
This project will utilize our extensive expertise in multimodal neuroimaging, gut microbiome assessment, and advanced bioinformatics and brain imaging analysis experience to study sex differences in structure, function and connectivity of brainstem regions and targeted brain networks and BGM interactions before and after a validated CBT intervention and a usual care control.

Aim A: Identify sex differences in the impact of CBT on the connectivity between brainstem nuclei and brain networks, and between these networks at rest and after provocation.
Background and rationale. Ascending noradrenergic and serotonergic systems originating in pontine nuclei play an important role in state-dependent regulation of interconnected brain networks, related emotional state, pain sensitivity and autonomic activity[58]. A dysregulation of these ascending systems has been extensively studied in preclinical and in psychiatric patients, and has previously been implicated in IBS pathophysiology.[3, 59, 60] Our preliminary data (**Fig. 3**) have demonstrated an association of plasma KYN levels with neuroplastic changes in these brainstem regions in IBS, and a remarkable reversal of these structural changes in CBT responders (**Fig. 7, left panel**).
Hypotheses and expected results. Hypothesis(H)A1: CBT, but not usual care will result in a *reduction* of various measures of functional and anatomical connectivity of brainstem nuclei with cortical brain networks, and between salience, emotional arousal and sensorimotor networks compared to the usual care, and this effect will be most pronounced in CBT responders. In responders, the pain threat task will show smaller threat responses in regions of the arousal and salience networks following CBT.
HA2. Female IBS patients will show larger alterations in the functional (resting and evoked responses) and anatomical connectivity of arousal and salience networks at baseline compared to men, and female responders will show the largest changes in these networks after treatment.
HA3. Changes in multisensory sensory sensitivity and declines in non-GI symptoms will be associated with the brain responses described above in brain stem and cortical networks.
Experimental strategy. Male and female participants randomly assigned to CBT or usual care will be scanned with the full set of neuroimaging protocols described in the Neuroimaging and Bioinformatics Core including the pain threat task before and after the intervention period. Female participants will all be premenopausal and scanned in the follicular phase of the menstrual cycle at both time points. The full set of clinical self-report questionnaires will be given at each time point.
Potential pitfalls and alternative strategies. Based on our preliminary (see above) and previously published results,[23] we expect that the clinical response from CBT will be robust with about a 60% responder rate. Dr. Lackner has successfully performed several trials with the same type of CBT in IBS patients. He will supervise the delivery of the CBT in the proposed study and has personally trained the therapist who will deliver the CBT. Should the response rate be either much higher or lower or vary widely between men and women leading to a decrease in power for the responder-based analysis we will look at response to therapy as a continuous variable with sex as a moderator to test the proposed hypotheses.

Aim B: Identify sex differences in the impact of CBT on gut microbiome
Background and rationale. Our preliminary data suggest that gut microbial relative abundances are altered by CBT in IBS responders. We expect that these microbial changes are associated with changes in microbial metabolites which may contribute to an attenuation or even reversal of pre CBT neuroplastic brainstem changes. It is not known if these responses are different in men and women with IBS. In this project, we will study the association of plasma metabolite changes in both men and women to CBT with brain measures during rest and during a provocative task aimed at activation arousal circuits.

Hypotheses and expected results. HB1: CBT compared to usual care will result in an increased abundance of Clostridiales taxa, a change in tryptophan metabolites, and possibly a reduction of the KYN/TRP ratio, and this will be most pronounced in CBT responders.
HB2: Female IBS patients will show greater alterations in microbiome parameters at baseline compared to men, and female responders will show the largest changes in gut microbiome measures after treatment.
HB3: Changes in microbiome including sex differences in these responses will be correlated with changes in brain stem and cortical brain imaging measures outlined above.
Experimental strategy. Male and female participants will be randomly assigned to CBT or usual care and changes in relative abundances of microbiota, microbial genes, bile acids, short chain fatty acids, and plasma metabolites after the intervention period will be determined.
Potential pitfalls and alternative strategies. If needed as described for Aim A, we can utilize continuous measures of response. In addition, we may also include exploratory analysis of alternative metabolites between responders and nonresponders.

<u>Aim C:</u> Identify clinical, brain and gut microbiome predictors of CBT outcomes
Background and rationale. There is currently very little data related to variables that may impact CBT outcomes in IBS. Our recent clinical data obtained in IBS subjects, as well as some preliminary brain imaging data obtained in UCPPS patients (summarized above), suggest that a patient phenotype that includes trait anxiety, and somatization, and higher relative abundance of the taxa Bacteroides in feces may be less responsive to treatment.
Hypotheses and expected results. HC1: IBS patients with a greater degree of alterations in brain network connectivity, including those with higher multisensory sensitivity, somatic co-morbidity and alterations in responses of brain stem and cortical networks during pain expectation will be less likely to respond to CBT.
HC2: Data-driven analysis of the broad range of pre-treatment variables will be able to identify specific subgroups more or less likely to show clinical benefit from CBT and any sex differences in these subgroups.
Experimental strategy. Clinical, brain imaging and metabolomic variables collected at baseline and before CBT treatment will be evaluated as predictors of treatment response and sex will be evaluated as a potential moderator of any significant relationships found. We will test the specific hypothesis in HC1 using logistic regression. We will also perform data-driven supervised analysis of the broad range of pre-treatment variables to identify which variables (i.e., clinical, brain, microbiome, sex) best predict response of treatment response.
Potential pitfalls and alternative strategies. Our initial predictors will be based on preliminary data including resting and provoked measures of activity in the emotional arousal and salience networks and connectivity between these networks with brainstem nuclei. If these a priori predictors are not confirmed, we will chose a more exploratory and hypothesis generating approach. Such an alternative approach may be justified given the limited prior data on prediction of response, and the lack of male subjects in previous studies.

PROTECTION OF HUMAN SUBJECTS

1. RISKS TO HUMAN SUBJECTS

a. Human Subjects Involvement, Characteristics and Design

Human subjects.
Study populations*: *IBS patients: 180 (50% male, 50% female) subjects with Irritable Bowel Syndrome (IBS) will be recruited in years 1-4 years to complete the studies. Estimates of data loss from drop out will yield samples of 100 completing CBT and 50 usual care. The larger sample size for CBT reflects the power needed to compare responders with non-responders within this group. Similar to our previous studies, the subject population will be composed of male and female IBS patients (all bowel habit subtypes) between the ages of 18-55. Female subjects will all be premenopausal and procedures will be run in follicular phase of their menstrual cycle. The diagnosis of IBS and bowel habit subtypes (IBS with diarrhea [IBS-D], IBS with constipation [IBS-C], IBS with mixed bowel habits [IBS-M]) will be made using ROME IV diagnostic criteria for IBS,[1] and no evidence of organic GI disease. Diagnosis will be confirmed by the study coordinator who is a nurse practitioner with longstanding IBS expertise. IBS subjects will complete the IBS-SSS, a validated multi-item instrument that measures IBS severity,[2] and must have a score ≥175 (at least moderate severity) and average pain in the past 1 week of at least 3 on a 0-10 point scale where 0=no pain and 10=worst imaginable pain to be eligible. Additional exclusion criteria for all subjects include active psychiatric illness over the past 6 months based on a structured clinical interview (MINI), or a current history of tobacco or alcohol abuse.

Medications: Exclusion criteria include use of 5-HT acting agents (e.g., antidepressants) that could affect TRP-related measures, antibiotics and probiotics in the past three months. Medications which alter GI motility and gastric pH will be stopped at least 72 hours prior to any physiologic test visit and during the baseline and treatment intervention. However, rescue medication of bisacodyl and loperamide will be given for prolonged constipation and diarrhea, respectively, during intervention periods but not baseline. To determine symptom severity, IBS subjects will complete the IBS-SSS,[2] a validated, well accepted tool to measure IBS severity. Total possible overall score is 0-500 with remission <75, mild 75-175, moderate 175-300, severe >300.[2, 3] Addition measures include one week electronic symptom diary (pain on a 0-10 NRS49 bloating 0-10 NRS, and stool frequency and form). Multisensory sensitivity and pain co-morbidity will be measured by the CMSI scales of Somatic Awareness and Sensory Sensitivity,[4] the Sensory Hypersensitivity Scale (SHS),[5] and the Chronic Overlapping Pain Conditions Screener (COPCs) based on the CMSI which includes symptom diagnosis for 10 common pain conditions and a body map of pain sites. Supportive questionnaires will include cognitive and affective measures related to chronic pain experience including the Pain Catastrophizing Scale, (PCS),[6] Hospital Anxiety and Depression (HAD) scale,[7] and the Early Traumatic Inventory-Self Report (ETI-SR).[8] and GI symptom anxiety (VSI)[9] will also be measured**.**

Inclusion for all IBS subjects: 1) Must meet Rome III criteria for irritable bowel syndrome, in the absence of red flag signs including unexplained weight loss, bloody stool, fever, or anemia. 2) If a woman of childbearing potential, must have been willing to use contraception to avoid pregnancy during the course of the study. Exclusion for all subjects: 1) Planned major medical intervention at 6 months (e.g., surgery) from the time of consent, or surgery in the past 6 months. 2) Presence of a significant and ongoing medical problem that would interfere with participation in the study or testing the study hypotheses (e.g., major heart disease, neurological disorders, inflammatory bowel disease, etc. 3) Presence of a major psychiatric diagnosis such as schizophrenia, Bipolar disorder, Post-traumatic Stress Disorder, or Obsessive Compulsive disorder. However, subjects with a history of DSMIV diagnosis of Anxiety or Depression, in whom symptoms are not active will be allowed but noted for post-hoc analysis. 4) Use of centrally acting medications that will interfere with the neuroimaging testing (e.g., opiate analgesics, antidepressants). 5) Left handed due to importance of laterality in brain imaging analysis. 6) Pregnancy or nursing. 7) Claustrophobia or other contra-indications for MRI (including ferrous metallic implants). 8) Current or past history of chronic pain syndrome in healthy controls; current or past

history of chronic pain condition in IBS patients that is predominant over IBS. 9) History of gastrointestinal surgery other than uncomplicated appendectomy or cholecystectomy.

In addition, Subjects with ferromagnetic implanted devices that might produce a safety hazard (e.g. infusion pumps, pace makers, aneurysm clips, etc.) will be excluded from the study. We will use as a guideline the manual "Magnetic Resonance: Bioeffects, Safety, and Patient Management, 3rd Ed., Frank G. Shellock, Ph.D. and Emanuel Kanal, M.D., Lippincott-Raven Pulishers" supplemented by the current information published on the International MR Safety Web Site. Pregnant women will be excluded from our study due to the unknown risk to the unborn fetus resulting from the exposure to low levels of magnetic fields from the MRI. Subjects with severe claustrophobia will also be excluded.

No ethnic minorities will be excluded.

b. Study Procedures, Materials, and Potential Risks

Study Procedures

Screening: Subjects will have an initial screening visit to obtain informed consent, confirm subject eligibility with history, physical exam and structured psychiatric exam, and perform screening detailed qualitative and quantitative dietary assessments).

Procedures:
1. Gut microbial profiles and signaling mechanisms: 16S rRNA sequencing, shotgun metagenomics, stool and plasma metabolomics with a particular focus on TRP metabolites (5-HT, kynurenine, indols), 2^{o}BAs, and SCFAs. Stool samples will be collected prior to MRI.
2. MRI imaging with a 3T magnet will be conducted on all subjects. Scanning will also include a pain anticipation task involving 2 trials of a painful but tolerable brief electrical stimulus to the abdomen. The scan will be 2 hours.
3. Minimal contact cognitive behavioral therapy (MC-CBT)

Materials
MRI image information will consist of images of the brain collected in the MRI scanner facility. Each scan will be given a unique code upon its acquisition, such that the subject does not have one unique code. The image transfer from MRI or from other sites to the CNSR will be sent via the existing secure medical system Ethernet. All patient information will be stored in a locked cabinet in the CNSR. This office will additionally be locked when not in use. The anonymized patient initials will be linked to the patient's full name on a separate document kept exclusively at the study site to protect patient confidentiality. Only the PI and authorized designates will have access. The source documents may be looked at by a representative from the Ethics Committee or a government regulatory agency, such as the FDA. Full name, actual patient initials and/or address of the patient, however, will not be disclosed outside the study site.

Potential Risks
There are minimal risks to the subject since all identifying information will be anonymously coded and protected by an appointed staff member of the CNSR. Venipuncture with intravenous catheter placement: when having the IV placed and blood drawn, subjects may experience some discomfort as a result of the needle prick. Some bruising or slight bleeding may occur. The blood draw will be performed by fully licensed and experienced medical personnel. Collecting a stool sample: This will be done privately by the study patients in their homes using kits provided to them by the study staff. There are no risks associated with self-stool collection other than discomfort from handling stool. Steps will be taken to minimize risks from the MRI scanner (as outlined below).

2. ADEQUACY OF PROTECTION AGAINST RISKS

a. Informed Consent and Assent
The screening visits for subjects enrolled into all studies will be performed at the UCLA CNSR Clinical Research Center. All prospective patients will be given the Informed Consent in advance (via email). If they are interested in participating in the study, they will be interviewed by one of the PIs and they will be given detailed written and verbal descriptions of the study protocol, the reason we are performing this study, and the potential risks to themselves should they decide to participate. Informed consent will be obtained by one of PIs after the subject reads the informed consent, can demonstrate understanding, and has all questions answered by IRB authorized study personnel prior to any study enrollment or procedure. Subjects will be given a copy of their Informed Consent Form.

b. Protections Against Risk
All identifying information as well as all historical and biological data will be anonymously coded and maintained under lock and key by an appointed trained member of the CNSR. All study procedures were performed by CNS staff members experienced in the performance of similar clinical studies and whom all have appropriate Human Subjects and HIPPA certifications. All study members involved in the imaging procedure maintain MRI safety certification. Well-documented protocol and safety limits are being adhered to and only trained personnel authorized by the PI operate the equipment.

The FDA Center for Devices and Radiological Health, released the document "Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices" on July 15, 2003. This describes their current evaluation of risk related to MRI. Notably, both software and hardware features of the instruments proposed in the current study prevent executing commands outside of a well-defined range defined as safe by the FDA. To minimize risks, we will use the following criteria:

- Field strength will be less than 4 Tesla at UCLA.
- Radiofrequency power deposition (measured as the specific absorption rate, or SAR) to the subject is limited by law, and by the instrument, to less than 3 W/kg in the head. Notably, human imaging with SAR of twice the limit has demonstrated to be safe in normal, healthy subjects.
- Any time rate of change of gradient fields (dB/dt) less than those sufficient to produce severe discomfort or painful nerve stimulation. Our lab is constrained to use gradient fields that do not produce detectable physiological sensation.
- A weighted root mean square (RMS) sound pressure level of less than 99dB with hearing protection in pace.
- Under no circumstances will our instrument be operated outside the ranges outlined above on human subjects.
- The small risk of metal objects being dislodged will be reduced by a thorough MR safety screening process.
- Ear protectors will be used to reduce any discomfort associated with scanner noise.
- The use of headphones and external view mirror will be used to minimize the potential discomfort of claustrophobia.
- Subjects will be given a "squeeze-ball" with which to alert the investigators should they experience any physical or psychological discomfort.
- The electrical stimulus used in the pain threat task has been used in multiple previous studies and is delivered by an FDA approved device. While it does generate some anticipatory anxiety during the 30 sec periods in which it might be delivered, participants are given experience with the stimulus before the scanning and choose the level so that it will not be overly stressful.

c. Vulnerable Subjects
This study does not include the recruitment of vulnerable populations as described by NIH.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO RESEARCH PARTICIPANTS AND OTHERS

The research proposed offers no direct benefit to the patients. The risks are very small. They received non-coercive compensation for the time required to complete the evaluations ($60 – 125) and for parking at UCLA ($12). The benefits of the study to others include advancement in our understanding of brain-gut-microbiome mechanisms underlying symptoms in IBS.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

IBS is a significant health care burden, affecting 9-11% of the American adults. IBS is a heterogeneous disorder in which all studies have demonstrated that only a subset of patients have a specific biologic abnormality or respond to a specific treatment. As there is no consistent diagnostic or therapeutic biomarker for IBS, symptom based classification is used for clinical trials and clinical practice. The proposed studies aim to identify biologically based subgroups within IBS. The potential exists that the knowledge gained as a result of this research may help in the future with respect to patient monitoring and the tailoring of treatments in patients with IBS. The low risks of the protocol are far outweighed by the potential benefit to provide clinicians with a powerful tool in which to monitor the patient.

5. DATA AND SAFETY MONITORING PLAN

The following procedures will be followed, in compliance with NIH requirements to ensure the safety of study participants and the validity and integrity of data. Confidential participant data will protected by assigning a unique numerical code to each patient, the key to which is kept in a locked filing cabinet in the CNSR database offices. The Program PIs will monitor adherence to the confidentiality rules, the quality of the data, their timeliness, and the correctness of their entry into computer files.

DATA AND SAFETY MONITORING PLAN

I. Overview

Cognitive Behavioral Therapy (CBT) is the most well researched and most effective treatment for IBS that targets the brain-gut-microbiome (BGM) axis, and preliminary data show that this therapeutic effect is associated with a reduction of brainstem connectivity with other brain networks. The increased prevalence of IBS in women, the higher rate of comorbid non-GI pain conditions, as well as the higher prevalence in female IBS of increased sensitivity to a variety of internal and external stimuli (multisensory sensitivity) suggest the presence of important sex differences in some of these BGM mechanisms. Research performed by the UCLA SCOR during previous funding has established an increased responsiveness of the CRF-Locus Coeruleus (LCC) system in female IBS subjects, suggesting that this noradrenergic brainstem system plays an important role in IBS pathophysiology. In addition, our earlier research has begun to identify clinical, functional and cortical brain mechanisms that may underlie these sex effects. Based on these preliminary data, the overall goal of this Project is to use CBT as a probe to study the relationship between specific disease-related alterations of the brain, the gut microbiome, and symptomatic outcome, and identify the role of sex differences in these relationships. We will study male and female IBS patients before and after CBT or usual care using the advanced neuroimaging and microbiome technologies of the overall SCOR to test the following: **Aim A.** will identify sex differences in the impact of CBT on the connectivity between brainstem nuclei and brain networks and between these networks at rest and after provocation. **Aim B.** will identify sex differences in the impact of CBT on gut microbiome parameters and **Aim C** will identify clinical, brain and gut microbiome predictors of CBT outcomes.

B. Adherence Statement

The Data and Safety Monitoring Plan (DSMP) outlined below will adhere to the protocol approved by the UCLA IRB and Clinical and Translational Science Institute (CTSI) Scientific Review Committee (SRC).

II. Adverse Events

A. Adverse Event Assessment

Anticipated adverse events for this study include symptom worsening due to the natural temporal course of IBS, although it is unlikely these symptoms will exceed beyond the normal intensity of the patients have typically experienced. In addition, anticipated possible adverse events include:

- Venipuncture with intravenous catheter placement: when having the IV placed and blood drawn, subjects may experience some discomfort as a result of the needle prick. Some bruising or slight bleeding may occur. The blood draw will be performed by fully licensed and experienced medical personnel.
- Collecting a stool sample: This will be done privately by the study patients in their homes using kits provided to them by the study staff. There are no risks associated with self-stool collection other than discomfort from handling stool.
- CBT: Talking about stress and problems can be distressing.
- Loss of confidentiality: Research personnel have procedures in place to de-identify all research data to minimize the chance that a loss of confidentiality will occur.
- MRI scans: There is no known radiological risk of MRI scanning. Subjects will be screened in a standard fashion for magnet sensitive substances (metal prosthesis, full body tattoo, etc.) prior to the study. Subjects may experience claustrophobia or anxiety during the scan, though subjects with pre-existing claustrophobia will be excluded. The overnight fasting for the imaging experiments may be unpleasant for some subjects, but they will be allowed to resume normal food intake after the procedure. The study medical and research staff is experienced in these settings and well able to assist subjects with any distress.

- The electrical stimulus used in the pain anticipation task has been used in multiple previous studies and is delivered by an FDA approved device. While it does generate some anticipatory anxiety during the 30 sec periods in which it might be delivered, participants are given experience with the stimulus before the scanning and choose the level so that it will not be overly stressful.

B. Evaluation of Adverse Events

To monitor for any anticipated and unanticipated adverse effects (as defined on the OHRP website), participants will be contacted weekly during the treatment intervention and will be monitored during study procedures and contacted within 24 hours following each procedure. Anticipated adverse events for this study include symptom worsening due to the natural temporal course of IBS and also those listed in the "Risks to Human Subjects" section above. The study will be halted for re-evaluation if two or more subjects have intolerable adverse effects that are judged to be due to the study procedures or interventions.

C. Reporting of Adverse Events

1. All adverse events will be reported to the PI and documented on study specific case report forms and entered into a computer-based log. The investigators have developed structured, detailed procedure for assessing and reporting adverse events in their human studies. An adverse report will be generated for each adverse event. This report will include a description of the event, how it was reported, timeline of the adverse event (when it occurred and its temporal relationship to any study procedure or treatment intervention, determination of attribution, and any treatment or hospitalization that was received for the adverse event. This procedure is consistent with best clinical practice.
2. Anticipated adverse events for this study include those listed in the "Risks to Human Subjects" section above. All unanticipated adverse events related or possibly related to the study will be reported to the PI within 24 hours. The PI and research team will report this to the IRB according to Human subjects protection policies and procedures.
3. The PI and Co-Is will review cumulative adverse events, early termination of study participation, and accrual on a monthly basis and report any issues requiring modification of the study or alteration of the risk: benefit ratio to the IRB immediately.
4. A summary of adverse events, study progress and protocol modifications will be included for IRB review in the continuing review report. Any action resulting in a temporary or permanent suspension of this study (e.g., IRB actions, or actions by the PI or co-investigators) will be immediately reported to the appropriate NIDDK program official.

III. Safety Review Plan and Monitoring

A. Justification of Sample Size

For the minimal contact CBT intervention: Preliminaries studies demonstrating CBT changes in mean abundance of Clostridiales order show strong effects (Cohen's d= 1.47). Moderate (d=.40) to large (d=.80) brain changes have been observed with CBT.[63-66] Based on two-tailed dependent t-test and an alpha of .05, 50 subjects will provide adequate power (80%) to detect an effect size difference as small as d=.40, if it exists, t(49)=2.01, noncentrality parameter=2.86.

B. Safety and Study Progress Reviews

This study does not require a Data Safety and Monitoring Board (DSMB) per the NIH criteria as it is not a Phase 3 trial, Phase 1 or 2 that involves masked interventions, a multicenter clinical study, a clinical trials of high risk intervention or an outcome assessment that is invasive or involves more than minimal risk, or a

clinical trial involving vulnerable populations that are more than minimal risk.

Some of these risks are considered to be minimal and others are considered greater than minimal risks; however, all risks are addressed in the protocol and consent form. The MPIs, Drs. Emeran Mayer and Lin Chang, will conduct oversight for the conduct of the study. They will meet weekly with the study coordinator and monthly with the Co-Is and other members of the research team. They will ensure that all eligibility criteria and consent requirements are met prior to a participant's participation on study, that retention of participants and protocol adherence are maintained and that all study procedures and adverse event reporting occur according to the IRB approved protocol. During the execution of the study, the PI and other members of the research team will monitor study progress and review interim analyses of outcome and safety data. Specific attention will be paid to the quality of collected data, patient recruitment and retention and reasons for dropouts. The MPIs will prepare annual reports that address all adverse events, judgments as to whether data collected has been conducted in a uniform, systematic manner, recruitment and accrual of participants, quality of data, and treatment adherence, whether the study is on track for completion and accomplishing stated aims, and if changes in study conduct are needed.

C. Protection of Participant Safety

Clinical trial patient safety will always be a priority. All subjects participating in these studies will be evaluated before entry into the study to assess for concurrent medical conditions. Even though the non-drug treatments featured in the proposed studies have a benign side effect profile for physical health problems, participants may experience fluctuations in symptoms including anxiety and depression during the course of study participation. Patients with active clinical levels of depression and or anxiety will not be ineligible to participate in the study. At pre-treatment baseline, during the acute treatment phase, and at follow-up visits, gastrointestinal (GI) symptoms and levels of anxiety and depression will be systematically assessed using psychometrically sound instruments and a structured psychiatric interview (MINI). Pregnant women will be excluded from the study. Urine pregnancy tests will be performed for all women with childbearing potential before any study procedure (e.g., MRI).

All study procedures will be performed by research members who are experienced in the conduct of similar clinical studies and have appropriate Human Subjects and HIPPA certifications. All study members involved in the imaging procedure maintain MRI safety certification. Well-documented protocol and safety limits are being adhered to and only trained personnel authorized by the PI operate the equipment. A physician will be available on call at all times.

All subjects participating in these studies will undergo a medical and physical examination before entry into the study to assess for concurrent medical conditions. The study doctor or nurse practitioner will determine if they are the best candidates for the study using the appropriate inclusion and exclusion criteria.

The blood draws and IV cannulations, and MRI administration will be performed by fully licensed and experienced medical personnel. Syringes and all material that had contact with blood will be disposed of accordingly to minimize the risk of injuries and prevent possible transfer of diseases. All samples will be labeled with the respective subject numbers. Samples will not be used to test for any disorders, either transmittable or genetic, that are not explicitly specified. Strict confidentiality will be maintained throughout the trial and in management of data by the use of initials and subject ID numbers on patient source documents, locked cabinets for patient related materials, and the use of unique subject ID numbers in databases. All individuals involved in the conduct of this study (including the PI, co-Investigators, nurses, technicians) are experienced in the tasks they will perform and in maintaining patient confidentiality. The subjects will be informed that they can discontinue the study at any time. Our collective experience indicates that the risks of any of the interventions or testing in this protocol are minimal and that serious complications are very rare. We feel these risks are justified given the need to identify subgroups of IBS patients susceptible to respond to the different treatment modalities and have a better understanding of the possible mechanisms behind IBS

development.

Therapists should be aware of pre-treatment baseline levels of distress based on clinical interview and data from psychological testing (e.g. Hospital Anxiety and Depression Scale, structured psychiatric interview, etc.) with the notion that elevated distress may signal more serious maladaptive coping behaviors (e.g. self injurious behaviors), and the value of ongoing monitoring of participants with high levels of distress through the acute treatment phase. Therapists should be encouraged to administer appropriate psychological testing instrument at each clinic visit to characterize levels of distress even if their administration falls outside of the testing schedule. Therapist should familiarize themselves with psychometric properties (e.g. cutoff scores for different levels of depression severity) of screening instruments. In addition to psychological testing, it is imperative for therapists to review the results of testing with patients concerning their symptoms (e.g. anxiety, depression) and treatment options if they are at imminent risk for suicide. To safeguard patient safety, the investigative team will follow Dr. Lackner's (Co-I) comprehensive suicide risk management plan that contains a specific list of actions for managing a suicidal participant (e.g., who to contact, what to document) which he used for his NIH funded CBT study in IBS. For patients whose distress is increasingly a significant source of impairment and interferes with their ability to undergo behavioral treatments, the PI may refer the participant for additional follow-up, additional therapy, and/or assessment for psychopharmacological intervention. Decision making should always be based primarily on what is in the best interest of patient safety. Depending on the situation, the changes may be temporary or continue throughout the study. In rare cases the experimental treatment may need to be discontinued, however participant would continue to be followed per Intent to Treat principle. Any decision to triage volunteer to another treatment facility should be communicated expeditiously to the referring MD and PMD within the constraints of HIPPA regulations. A list of local mental health facilities to which patients can be expeditiously referred for mood stabilization or other mental health problems that are beyond the scope of the study behavioral treatments.

D. Stopping Rules

The project is expected to last 5 years. It is unlikely that the event will be terminated early, for either negative or positive reasons. There is no universally agreed upon method for determining when to stop a behavioral trial. Stopping rules based on sample size and power has not been adequately delineated. However, evidence that any of the interventions produce clear evidence of harm and review of an interim futility analysis which shows no likelihood of demonstrating a treatment benefit would also lead to early termination of the study. The study will be halted for re-evaluation if two or more subjects have intolerable adverse effects that are judged to be due to the study procedures or interventions.

IV. Informed Consent

The screening visits for subjects enrolled into all studies will be performed at the UCLA CNSR Clinical Research Center. All prospective patients will be given the Informed Consent in advance (via email). If they are interested in participating in the study, they will be interviewed by the PI who is a gastroenterologist and they will be given detailed written and verbal descriptions of the study protocol, the reason we are performing this study, and the potential risks to themselves should they decide to participate. Informed consent will be obtained by the PI after the subjects have had all their questions answered and before entry into the study.

V. Data Quality and Management

A. Review of Data Collection Forms

The MPIs will have weekly meetings with the study coordinator and review progress of the study recruitment, quality and integrity of the data collected in the research records, the accuracy of the data submitted and to determine that all processes and/or regulatory requirements are being met. The MPIs will also meet with key personnel and staff on a monthly basis to review the same information as above.

The MPIs and investigative team will allow representatives of NIDDK to periodically monitor, at mutually convenient times during and after the study, all CRFs and corresponding source documents for each participant. These monitoring visits provide the opportunity to evaluate the progress of the study and to inform NIDDK of potential problems. The research study coordinator will assure that submitted data are accurate and in agreement with source documentation; verify that study treatments are properly provided; verify that participants' consent for study participation has been properly obtained and documented; and confirm that research participants entered into the study meet inclusion and exclusion criteria.

B. Data Integrity and Protection of Databases

All participant information, and even the fact that an individual is participating in the study, is considered confidential. This confidentiality is assured through several mechanisms. First, each participant is assigned an anonymous study ID, which is then used on all study forms. Second, all study forms, and paper records that contain participant information (e.g. consent forms, address lists, phone lists) are kept in secured, locked areas when not in use. In addition, such materials, when in use, are kept away from public scrutiny. Materials and specimens that can be discarded are destroyed. Third, access to all participant data and information, including laboratory specimens, is restricted to authorized personnel. In the case of computerized data, this restricted access is assured in several ways. The data are maintained on the Data Manager's computer that is password-protected and backed up daily. Only the PI and authorized personnel are granted access to the data, and, second, this access is further restricted by password protection. When the study database is made available to the investigators, it does not include actual identities and contact information of participants. Such information is retained in the recruitment log for use in the event that future follow-up of the study participants is necessary. Finally, participants are not identified by name in any reports or publications, nor are data presented in such a way that the identity of individual participants can be inferred. Any presentation or publication of the data will be done in aggregate fashion without identifiers.

All members of the research team are required to complete a confidentiality certification procedure upon employment. Policies regarding the confidential nature of the data collected, processed and stored are explained to all personnel who must then sign a "confidentiality certification" before being allowed access to confidential information. The MPIs will continually reinforce the need for careful and confidential handling of data at staff meetings and trainings.

VI. Confidentiality

Privacy in the context of this study includes confidentially of data and personal information at the participating sites and in the handling and reporting of data. It also includes discretion of the part of the clinical center staff and arrangements or physical privacy during interviews and examinations. The research team is responsible for ensuring physical privacy of participants and ensuring that data are stored in a secured area accessible only to study staff. These provisions and arrangements will be monitored by the MPIs.

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