Research Summary Template

1. Protocol Title:

The Probiotic Study: Using Bacteria to Calm your Mind

2. Purpose of the Study:

We seek to 1) conduct the first open-label trial of probiotics in young children, 2) examine the effects of probiotic treatment on the reduction of anxiety and abdominal pain, 3) examine changes in stress-reactivity as a potential mediator of treatment effects, 4) assess the feasibility and efficacy of this treatment, and 5) elucidate the pre- and post-treatment composition of enteric microbiota in the middle-lower GI tract as a potential mediator of treatment effects.

3. Background & Significance:

Approximately 10% of children presenting to primary care facilities complain about abdominal pain, the most frequent symptom in gastrointestinal (GI) diseases (Peery et al., 2012; Ramchandani, Hotopf, Sandhu, & Stein, 2005). Annual costs for the diagnosis and treatment of GI diseases in the US have been estimated at $142 billion in direct and indirect costs (Peery et al., 2012). In addition to the direct costs related to GI disease is the added burden of comorbid psychiatric illness. When present, an anxiety disorder can significantly prolong the course and increase the burden of GI disease, which in turn can increase symptoms of anxiety (Shelby et al., 2013).

In the US, 28.8% of the population will receive a diagnosis of an anxiety disorder during their lifetime and 5-8% of 5-year olds meet diagnostic criteria (Kessler, Berglund, Demler, Jin, Merikangas, & Walters, 2005). Given the high rates of comorbidity and synergistic vulnerability imposed by GI disease and anxiety disorders, understanding and treating core processes that contribute vulnerability to both disorders may significantly alter the developmental course and outcome. This transdiagnostic focus is consistent with recent emphases of the NIMH, part of the Research Domain Criteria (RDoC) initiative (Cuthbert & Insel, 2013).

One barrier to treatment development is that the biological mechanisms that contribute to enhanced abdominal pain and/or anxious experience in children are unknown. Some studies show that interventions that directly alter gut microbiome composition (e.g., as in the use of probiotics), not only alter pain behavior, but also decrease anxious behavior in animal models (Collins, Verdu, Denou, & Bercik, 2009). In adult human studies, the gut microbiota is altered in individuals with irritable bowel syndrome, and symptoms may be relieved by probiotic consumption (Jeffery, O'Toole, Ohman, Claesson, Deane, Quigley, & Simren, 2012; Rajilic-Stojanovic, Biagi, Heilig, Kajander, Kekkonen, Tims, & de Vos, 2011; Saulnier et al., 2011; Whorwell et al., 2006).

Stress is one unifying biological mechanism that influences anxiety and GI pain. Heightened stress reactivity is a transdiagnostic process along the anxiety spectrum that is also associated with GI disease. Heightened stress reactivity is defined as heightened reactivity (e.g., increased autonomic reactivity and subjective perceived threat) to innocuous stimuli perceived as threatening, stronger physiological reactivity to noxious stimuli, and a slower return to baseline following stress provocation.(Chida, Sudo, Sonoda, Hiramoto, & Kubo, 2007). Elevated stress reactivity is also a marker of HPA axis dysregulation.(Kryski, Smith, Sheikh, Singh, & Hayden, 2013). Due to evidence that shows stimulation of the HPA axis, the

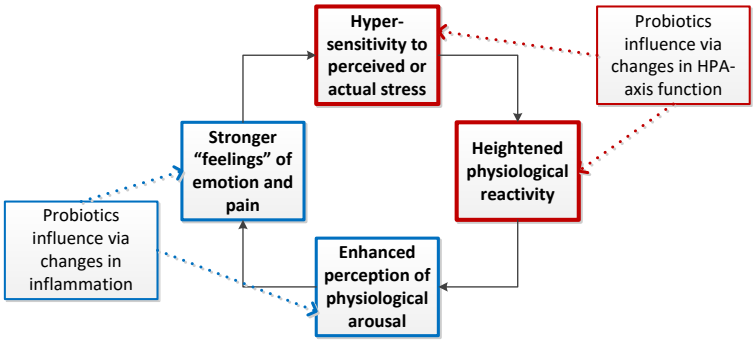


Figure 1. Red borders signify heightened stress reactivity and blue lines signal heightened visceral sensitivity. Probiotics are proposed to affect stress reactivity via changes in HPA-axis function and affect visceral hypersensitivity via changes in inflammation.

psychosocial stress induction task, Trier Social Skills Task is a justifiable mean of measuring stress reactivity (See Design and Procedures)(Gunnar, Talge, & Herrera, 2009a). Given the nature of the autonomic response associated with stress reactivity, heart rate variability and cortisol changes will be collected pre and post to evaluate the effectiveness of the probiotic treatment.

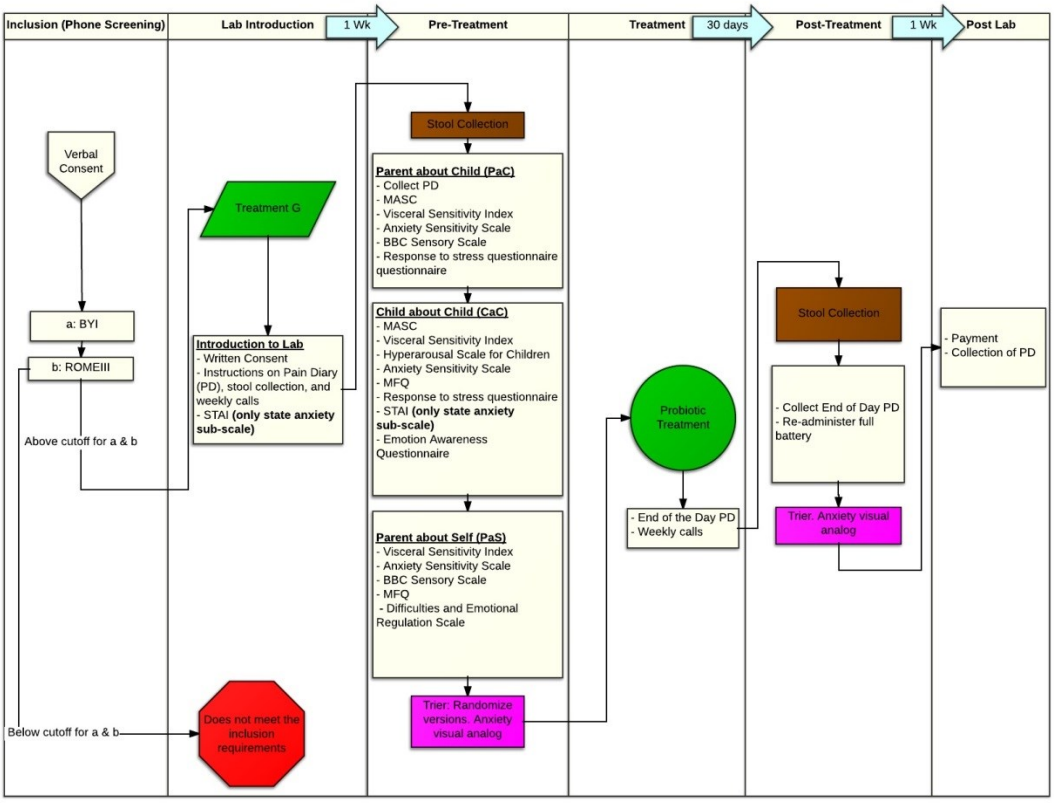
Additionally, HPA axis dysregulation has important implications for the development of the gut microbiome as will be discussed. A potentially related cross-diagnostic process is visceral hypersensitivity, subjective sensitivity to changes in visceral organs (e.g., an individual who readily senses changes in gut motility) (Azpiroz, Bouin, Camilleri, Mayer, Poitras, Serra, & Spiller, 2007; Bradesi et al., 2004; Mayer, Bradesi, Chang, Spiegel, Bueller, & Naliboff, 2008; Theodorou, 2013) In fact, such sensitivity to visceral change can become a subsequent cue for intensified reactivity in a vicious cycle (Figure 1). Given the early onset and chronic course of anxiety and GI disorders, an intervention that can impact transdiagnostic processes of stress reactivity and visceral hypersensitivity would be transformative. In the following section, we discuss how understanding communication between the gut and brain and the influence of the gut microbiome on such communication may provide key intervention mechanisms.

Therefore, alternations in the gut microbiome may specifically impact the core processes relevant for anxiety and GI disorders. Further, such changes may be evidenced behaviorally and physiological via improvement in stress reactivity (more sensitive cortisol dynamics in response to a lab-based stressor). Study of proposed biological and behavioral mechanisms of new treatments for mental disorders is consistent with the recent strategic plan of the NIMH emphasizing more mechanistically-informed intervention development.

4. Design & Procedures:

In this open-label trial, self-report measures and a laboratory task will be collected from children or their primary caregivers prior to and following a 30-day probiotic administration (See Figure 2).

Figure 2: Study design and timeline. Acronyms are explained in the subsequent paragraphs.



Inclusion (Phone Screening): Parents of study participants will provide verbal consent over the phone prior to answering our screening questions about their children's abdominal pain and anxiety (see Telephone Consent for Screening). Abdominal pain will be assessed with questions taken from the ROME III questionnaire, and anxiety will be assessed with the Beck Youth Inventory (BYI). Participants below cutoff for both parameters will meet the exclusion criteria whereas those above cutoff will be given the probiotic treatment (Green Group in Figure 2). Participants who have immune disorders will automatically be excluded from this study.

1st Lab Visit (Introduction to the Study): Parents and children (dyads) will be invited to come to our lab for an introductory session and provide written informed consent. The researcher will explain specific details about the study as well as how to collect stool samples and complete the daily Pain Diaries. After dyads consent to participate, they are given the EasySampler Stool Collection Kit and Pain Diaries to be collected by the researcher in the next visit in one week. Children will also fill out the State Scale of the State-Trait Anxiety Inventory (STAI) to obtain a measure of current anxiety.

The researcher will specifically explain stool sample collection procedures as follows:

i. Please follow the instructions provided in the EasySampler® Stool Collection Kit to ensure an easy and hygienic stool collection and proper labeling.
ii. Collect samples either the day before or the day of a lab session. The closer the stool collection is to the delivery time the better.
iii. Place the tubes containing the samples in your freezer as soon as possible after collection.
iv. Bring the tubes on your appointment day.
v. You're done!

The EasySampler® Stool Collection Kit manual instructs to:

1. Follow instructions provided in the package
2. Handle and label collection containers accordingly
3. Use disposable gloves for stool collection
4. Tape EasySampler (paper device) onto the toilet rim as shown below:

5. Collect stool sample accordingly
6. Remove disposable gloves and flush EasySampler

For more information, refer to EasySampler® Stool Collection Kit manual and/or visit: https://www.alpco.com/store/easysampler-stool-collection-kit.html.

1-Week Assessment Period (Light Blue in Diagram).
Participants will complete a 1-week worth of a daily pain diary prior to treatment (or the control period) and for another week post-treatment. One -day prior to the 2nd lab visit, parents will have their children take a stool sample to bring with to their laboratory visit.

2nd Lab Visit (Pre-Treatment): One week later, dyads will return to the laboratory and will provide their stool samples and completed Pain Diaries. Stool samples will be stored in a -20°C freezer at the Duke Center for Developmental Epidemiology, in Suite 24-E, for less than 24 hours before being transported to Seed Laboratory located at Edwin L. Jones Building, Room 437, Research Drive, DUMC, Durham, NC 27710. Subsequently, dyads will fill out a full battery of diagnostic and self-report measures (See Table 1). Then, each child will participate in the Trier Social Skills Task (TSST). The Trier Social Skills Task has been designed to induce psychosocial stress in humans, and has been used in as early as 7 year-old children (Gunnar, Talge, & Herrera, 2009b). This task has shown evidence of psychosocial stress induction in humans via stimulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis as measured by changes in cortisol dynamics. This task is divided in several sections, including a speech preparation period, followed by the presentation of the speech and subsequent arithmetic problem solving. Pre and post resting periods precede and follow the task respectively. Physiological responses such as heart rate and changes in cortisol are collected throughout the whole experiment. The lab task lasts approximately 20 minutes, however, completion of questionnaires, salivary sampling, and the lab task combined will take about an hour and a half (Buske-Kirschbaum et al., 1997; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Jansen et al., 2000;Krishnaveni et al., 2014).

There will be two between-subject and within-subject TSST randomized versions, such that all individuals participate in both versions: one version at pretest and the other at posttest. Each version contains a different story lead. For more information on the story leads, refer to the TSST protocol. Both versions are followed by an arithmetic problem (see TSST protocol). Saliva samples will be collected 20 and 1 minute before TSST, 9 minutes before public speaking (i.e. at the end of preparation period), immediately after math problem, and at 20 minute intervals after TSST for 60 minutes.

TABLE 1: List of diagnostic and self-report measures
Parent about Child (PaC)
Daily Pain Diary
MASC
Visceral Sensitivity Index
Anxiety Sensitivity Scale
BBC Sensory Scale Questionnaire
Response to Stress Questionnaire
Child about Child (CaC)
MASC
Visceral Sensitivity Index
Hyperarousal Scale for Children
Anxiety Sensitivity Scale
MFQ
Response to stress questionnaire
STAI (state anxiety sub-scale)
Emotion Awareness Questionnaire
Parent about Self (PaS)
Visceral Sensitivity Index
Anxiety Sensitivity Scale
BBC Sensory Scale
MFQ
Difficulties and Emotional Regulation Scale

Treatment: Participants will be given a 30-day supply of an over-the-counter Probiotic Nutritional Supplement, Culterelle®, taken as directed per dosing instructions for children, 1 capsule/packet per day (see Figure 3 for product labeling). This administration of probiotics will be supervised by Dr. Richard Chung, MD. One of our staff members will give weekly calls to the parents, every seventh day of treatment, for four weeks, to assess tolerability address questions or concerns, and encourage compliance (See Tolerability Monitoring Script).

Caregivers and children will continue to complete an abbreviated daily pain diary that is completed at the end of each day and that assesses abdominal pain, anxiety, and other somatic symptoms.

3rd Lab Visit (Post-Treatment): After the 30-day treatment period, children will again be asked to collect a stool sample, and the questionnaires, and laboratory task will be repeated. Dyads will be given another week of daily pain diaries to complete.

Figure 3

Supplement Facts

Serving Size: One (1) Packet

	Amount Per Serving	% Daily Value ages 1-3	% Daily Value 4+ years of age**
Total Carbohydrate	1 g	†	<1%
Vitamin C	2 mg	5%	4%
Lactobacillus GG	5 billion CFUs***	†	†

** Percent Daily Values are based on a 2,000 calorie diet. †Daily Value not established.

Other ingredients: Mannitol, inulin, sucrose, maltodextrin, sodium ascorbate, and silicon dioxide.
Distributed by i-Health, Inc.
55 Sebethe Drive, Cromwell, CT 06416

Contains **none** of the following: added colors, preservatives, dairy, lactose, milk, yeast, gluten or soy.

Supplement Facts

Serving Size: One (1) Tablet

	Amount Per Serving	% Daily Value 3 years of age	% Daily Value 4+ years of age**
Calories	0		
Total Carbohydrate	<1g	†	<1%
Sugar	0	†	†
Vitamin C	5mg	15%	8%
Lactobacillus GG	5 billion CFUs***	†	†

** Percent Daily Value based on a 2,000 calorie diet.
† Daily Value not established.

Other ingredients: Xylitol, sucrose, microcrystalline cellulose, stearic acid, vegetable juice (color), maltodextrin, sodium ascorbate, natural berry flavor, magnesium stearate, citric acid, malic acid, silica.
Distributed by i-Health, Inc. 55 Sebethe Drive, Cromwell, CT 06416

Contains **none** of the following: synthetic colors, preservatives, dairy, lactose, milk, yeast, wheat, gluten or soy.

Follow-up: Participants will complete one week of PDs. At the time of completion, they will turn in their PD and will receive their final compensation.

5. Selection of Subjects. We will recontact participants who are now between the ages of **9 years old and 13 years old** who were part of a longitudinal study of preschool anxiety with protocol number Pro00008503 (See eligibility criteria below). To be eligible, participants need to meet the criteria for functional abdominal pain as specified by the Rome III criteria and depicted in Table 2. They also have to be above cutoff (i.e. TS>= 55) for BYI which can be determined by finding the corresponding raw score, gender, and age on Appendix A in the BYI manual:

- TS >= 55 represent mildly elevated to extremely elevated anxiety; therefore, eligible.
- TS <55 not eligible

Participants with immune disorders, and taking immunosuppressive medication (other than inhaled corticosteroids), will be automatically excluded from this study. Participants who have taken probiotics and antibiotics in the past month will also be excluded. Those with medical causes of abdominal pain (e.g. inflammatory bowel disease), and a mental disorder, other than an anxiety disorder, will be excluded. Those who have taken anxiolytics in the past year will be excluded as well.

Table 2. Scenario #1	**Criteria for inclusion**
A. In the last 2 months, how often did your child have pain or an uncomfortable feeling in the upper abdomen above the belly button?	If ≥ 8 (or If ≥ 1 plus impairment ≥ 25% of the time)
B. In the last 2 months, how often did your child have pain or an uncomfortable feeling in the area around or below the belly button?	If ≥ 8 (or If ≥ 1 plus impairment ≥ 25% of the time)

Eligibility Criteria for the study "Preschool Anxiety Disorders in Primary Care," Pro00008503

Inclusion/exclusion criteria for the screened sample (n=4,410)
Screening inclusion criteria will be (1) a child who is between 24 and 71 months old, (2) the presence of a parent/legal guardian at the clinic visit who speaks English well enough to consent to participate in the screening, (3) the child's attendance at the Duke pediatric clinic during a screening period. Exclusion criteria will be (1) lack of a parent with adequate fluency in English to complete the interview, and (2) the index child being known to have mental retardation (IQ < 70), autism, or other pervasive developmental disorders. Parents of children younger than 3 years of age will be asked to complete the Modified Checklist for Autism in Toddlers (M-CHAT), while parents of children 3 years of age and older will be asked to complete the Social Communication Questionnaire (SCQ). Any positive questionnaire would be followed up with a phone interview that used items from the Autism Diagnostic Interview (ADI) to determine whether the behavior is truly atypical. Exclusion for autism or other PDD will be based on the results of the screen and telephone follow-up; (3) sibling already enrolled in the study. If the parent brings more than one child between the ages of 24-71 months old to see the pediatrician, the recruiter will consult pre-computed random selection tables to select which child to enroll in the study.
Inclusion criteria for screen-stratified in-home interviewed sample (n=929)
Children who score 4 or more on the narrow band anxious/depressed syndrome scale of the CBCL1 ½ -5 will be recruited to participate in the study (N=706). We will randomly select 7.9% of children who score less than 4 on the anxious/depressed scale (N=223).
Inclusion criteria for nested case-control sample (n=500)
We will run the diagnostic algorithms on data from the in-home assessment to determine whether the child meets diagnostic criteria for any DSM-IV anxiety disorder (SAD, GAD, simple phobia, social phobia, PTSD, and/or selective mutism), and attempt to recruit all children with an anxiety disorder. We expect to recruit 250 children who meet these criteria, 240 from the screen positive group and 10 from the screen negative group. We will also randomly select 7.9% of children who do not have an anxiety disorder (N=37 from the screen highs, and 223 from the screen lows). We will ask the parent who completed the in-home assessment to complete the laboratory assessment with his/her child.

6. Subject Recruitment & Compensation:

We will recruit 40 children who are between 9-13 years old, both female and male, above cut-off for anxiety and abdominal pain. Potential participants will receive a letter/e-mail from Helen Egger, principal investigator for the pre-school anxiety study (Pro00008503), in which they will find a phone number they can call to opt in or opt out of being re-contacted for future studies. Those who call expressing interest in the study, will be screened to determine their eligibility. The contact information of those who opt out will be deleted immediately.

We will conduct a DEDUCE search using the terms "anxiety" and "abdominal pain" within the case load of Dr. Gary Maslow. Potential participants that meet these search criteria and are verified by Dr. Maslow as being appropriate for the study will receive a letter and/or email from Dr. Maslow informing the parent that their child may be eligible. Interested parents will notify the investigators by phone or email and a telephone screening call will be scheduled.

Lastly, we will start recruitment efforts at the Duke Children's Primary Care Southpoint, where our research assistant, Adam Kiridly, will introduce our study to parents who agree to learn more about it. Parents who show interest will be asked to complete a short contact form for phone screening.

Those who score below cut-off for anxiety and abdominal pain will be excluded. Each child-parent dyad will receive a compensation of $190.00 USD. The compensation amount will be broken down as follows:

Compensation Breakdown

1st Lab Visit (DPD)	$20.00
1Wk PD	10.00
2nd Lab Visit (Questionnaires and Physiological Measures)	40.00
Treatment (Probiotics)	30.00
Bonus	10.00
3rd Lab Visit	30.00
1WK PD	10.00
Gift Card/Bonus	40.00
Total	**$190.00**

7. Consent Process – see Section 14 of the e-IRB submission form and complete the questions in that section. – consent from parent and assent from child

Samuel Marsan, the project coordinator, will be conducting the consent process with prospective participants at the Duke Center for Eating Disorders, which is physically located at the Duke Center of Developmental Epidemiology. The consent process is expected to take about 10-20 minutes unless parents and children need more time to ask questions and think about participation. Participants who are below the age of 12 years of age will provide verbal assent; whereas, those who are 12 years of age or older will sign the consent form should they decide to participate in the study. Parents or legal guardians are also required to sign the consent form if they decide to participate in the study.

In order to protect the privacy of prospective participants, the consent process will take place in a laboratory at the center. The laboratory is accessible to Duke staff only. Study documents will be stored under three locks in the project coordinator's office. After consent is signed, participants will be given the PI contact information in case they have more questions after the study. The project coordinator has been trained in the consent process, and will follow good clinical practice guidelines

to minimize the possibility of coercion or undue influence. Participants who do not read, are blind, or who do not read/understand English will be excluded from the study.

8. Subject's Capacity to Give Legally Effective Consent:
Children who are below 12 years of age will provide assent and those who are 12 years or older will sign the consent form.

9. Study Interventions:
We will use Culturelle®, a probiotic composed of the micro-organisms Lactobacillus GG. This formulation has been used for the treatment of gastrointestinal inflammation in numerous clinical trials. Dosing will follow the manufacturer's recommendation for children (1 capsule/packet per day), and will be monitored by Dr. Chung. Weekly side effects and clinical changes will be monitored by both study therapist and caregiver using the Children's Global Assessment Scale and the Clinical Global Impression Scale (Severity, Improvement, and Efficacy)

10. Risk/Benefit Assessment:
Physical risks **-**There are no known physical risks to the child or parent associated with this proposed study. However, as we are recruiting children with gastrointestinal symptoms, there is the possibility that children's gastrointestinal problem will worsen due to the natural course of their disorder. The following steps will be taken with children whose abdominal symptoms worsen during the course of the study.
1. They will be referred for additional medical management.
2. Any medical management they receive will be documented.
3. They will be allowed to continue in the study so that they continue to receive psychological services.

Psychological risks –Assessment Risk: The parents may experience some psychological discomfort talking to the interviewer about some aspects of their lives or their children's lives. Children might find the observational assessments frustrating, disappointing, scary or boring. To address this risk, we will inform and reiterate that participants are free to refrain from answering any question that makes them uncomfortable.

Intervention – There are no known risks from taking Probiotics, a dietary supplement that attempts to increase the quantity of naturally-occurring healthy gut bacteria. The active cultures found in Culterelle® have been added to many food products available on grocery shelves (e.g., yogurt). Some of the side effects of taking this supplement may include mild bloating, gas or digestive gurgling when you first begin taking Culturelle. This is normal due to the changing environment in the digestive system and should go away within a short period of time.

Social risks - There are no known social risks.

Legal risks -There is a risk that study data might be subpoenaed for legal purposes. We will obtain a Certificate of Confidentiality from the FDA which includes the protections the certificate affords as well as the limitations and exceptions of the protection. In addition, except under circumstances covered under the mandated child abuse reporting laws, and/or situations in which the child and/or a caregiver is judged clinically to be a danger to themselves or others, no information about the child or family will be shared with any individual or agency without prior written consent.

Other risks - Participants will be informed of the federally mandated reporting laws for child abuse and neglect, verbally and in the written consent form. Specifically, the consent form will read: "This protection, however, does not prohibit the investigator from voluntarily reporting information about suspected or known sexual or physical abuse of a child or a subject's threatened violence to self or others. If the researchers learn that you or someone with whom you are involved is in serious danger

or harm, they may inform the appropriate agencies". Therefore, a serious, though rare, risk to families is disruption of the home based upon the severity of the abuse or neglect disclosed.

Potential Benefits. The project has the potential for direct benefit to the participants in the form of decreased abdominal pain, anxiety, and impressed capacity to cope with stress. In light of these potential benefits, we deem that the magnitude and low likelihood of risk is small relative to the magnitude of potential benefit.

11. Costs to the Subject: none
Costs to participants are their time and effort. There are no expenses associated with this study.

12. Data Analysis & Statistical Considerations:
Statistical Methods

In this pilot study, we have established stress reactivity, anxiety and abdominal pain as our primary endpoints. A post-hoc one-tailed power analysis will be conducted to determine the probability in which a probiotic treatment can significantly reduce anxiety and abdominal pain. Tests of hypotheses will be based on standard binomial models for proportions and Student's t-test or standard ANCOVA regression models as appropriate for continuous measures; ordinal data not meeting assumptions of normality will be analyzed using nonparametric Wilcoxon Mann-Whitney procedures or, in some cases, generalized linear regression (Poisson, logistic) models. Subjects who are below cutoff in our measures of anxiety and abdominal pain will be excluded from the study

Cortisol. Several steps will be taken to increase the accuracy with which children's baseline cortisol levels were indexed. All visits will begin between 1200 h and 1530 h to minimize the effects of diurnal variation on cortisol samples.(Gunnar et al., 2009b) Children will refrain from eating or drinking for a half hour prior to the visit to minimize the influence of food/drink on cortisol assays. Children will first play quietly with the experimenter for 30 minutes followed by a baseline salivary cortisol sample and the stress task described above. Following the stress task, the child and experimenter will resume quiet play while the remaining saliva samples are obtained. Children will chew on a 2-inch absorbent cotton dental roll until wet with saliva, which will be expunged into a micro tube and frozen until assayed in duplicate using an expanded range, high sensitivity, salivary cortisol enzyme immunoassay kit (Salimetrics, PA, USA). Standard curve and concentration of unknown samples will be generated according to manufacturer's instructions using a 4- parameter sigmoid minus curve fit.

Heart rate. We will measure heart rate continuously starting at 20 minutes before and ending at 60 minutes after TSST. We will use Garmin vivosmart HR, a wrist heart rate monitor , to collect this data. Heart rate variability will be analyzed pre and post treatment.

Data Extraction of Enteric Gut Microbiome. We will utilize next generation sequencing of stool ribosomal DNA to elucidate the enteric microbiota of children with anxiety and abdominal pain. We have prior experience with all of the described methods herin.(LaTuga, Ellis, Cotton, Goldberg, Wynn, Jackson, & Seed, 2011) Total DNA will be isolated from stool using a bead lysis protocol (Zymo Research). The V3 region of 16s rDNA will be amplified by polymerase chain reaction (PCR) using multiplexing primer pairs. Pooled amplicons will be sequenced on the Ion Torrent PGM platform in the Duke Genome Analysis core facility of the Institute for Genome Sciences and Policy. The sequences will be de-multiplexed and analyzed through the Qiime and Mothur pipelines to make taxonomic assignments and determine alpha and beta diversity.(Caporaso et al., 2010; Schloss et al., 2009)

Data Analysis of Enteric Gut Microbiome. Intra- and inter-individual statistical comparisons will be performed using the Unifrac metric combined with Kuskal Wallis testing and principle coordinate

analysis. Analyses will be performed to identify potential correlations between specific taxa and clinical pain and anxiety symptoms.

Statistical Considerations. Because of limited sample size and the iterative nature of intervention development, criteria for success at the pilot phase are based primarily on clinical rather than statistical criteria. We have established goals for compliance, completion, and reduction in physical and psychological distress aimed at establishing the feasibility and efficacy of the intervention. We carefully considered statistical constraints of pilot investigations by framing outcomes based on clinical but not statistical significance.

13. Data & Safety Monitoring:

DATA AND SAFETY MONITORING PLAN:
Data will be collected in the form of electronic forms collected via Qualtrics and the Duke owned and approved Redcap® database management system. Ensuring the integrity and confidentiality of the data is an essential part of safeguarding the rights of subjects. The Center for Developmental Epidemiology, from whose offices the study will be conducted, has instituted a series of checks at several levels of data handling, to maintain its quality and confidentiality. (1) Interviewers, many of whom have years' experience in survey work, will be given extra training in the importance of confidentiality in psychiatric interviewing. Like all study personnel, they will promise in writing to maintain that confidentiality. (2) After collection, coding, and review for accuracy by the project coordinator, data are entered into computer files at our offices at The Center for Developmental Epidemiology. The protocol, in both its physical (paper and pencil) and electronic (entered data forms), will be identified by number only. From this point it will not be possible to trace it back to an individual except by linking with a separate computer file that includes both the identification number and the individual's identity.
In accordance with federal regulations the PI will monitor for, review, and promptly report to the IRB, appropriate institutional officials, sponsor, coordinating center and the appropriate regulatory agency head all unanticipated problems involving risks to subjects or others that occur in the course of a subject's participation in a research study (45 CFR 46.103(b)(5)(i) and 21 CFR 56.108(b)(1)), and all reportable AEs will be submitted per the DUHS IRB policies; available at: (http://irb.duhs.duke.edu/modules/irb_pols/index.php?id=2)

ADVERSE EVENTS MONITORING:
In accordance with federal regulations the PI will monitor for, review, and promptly report to the IRB, appropriate institutional officials, sponsor, coordinating center and the appropriate regulatory agency head all unanticipated problems involving risks to subjects or others that occur in the course of a subject's participation in a research study (45 CFR 46.103(b)(5)(i) and 21 CFR 56.108(b)(1)), and all reportable AEs will be submitted per the DUHS IRB policies; available at: (http://irb.duhs.duke.edu/modules/irb_pols/index.php?id=2)

All adverse events (AEs), whether observed by the Investigator, elicited from the participant or volunteered by the participant, and whether ascribed to the drug or not, will be recorded. This will include the following: a brief description of the event, the date of onset, the date of resolution, the duration and type of the event, the severity, contributing factors and any action taken with respect to the study drug.

For each adverse event, the relationship to the study drug will be recorded as one of the choices on the following scale:

DEFINITE Causal relationship is certain (i.e., the temporal relationship between drug exposure and the adverse event onset/course is reasonable, there is a clinically compatible response to de-challenge, other causes have been eliminated and the event must be definitive pharmacologically or phenomenologically using a satisfactory re-challenge procedure if necessary).
PROBABLE High degree of certainty for causal relationship (i.e., the temporal relationship between drug exposure and the adverse event onset/course is reasonable, there is a clinically compatible response to de-challenge [re-challenge is not required] and other causes have been eliminated or are unlikely).
POSSIBLE Causal relationship is uncertain (i.e., the temporal relationship between drug exposure and the adverse event onset/course is reasonable or unknown, de-challenge/re-challenge information is either unknown or equivocal and while other potential causes may or may not exist, a causal relationship to the study drug does not appear probable).
UNLIKELY Not reasonably related, although a causal relationship cannot be ruled out (i.e., while the temporal relationship between drug exposure and the adverse event onset/course does not preclude causality, there is a clear alternate cause that is more likely to have caused the adverse event than the study drug).
NOT RELATED No possible relationship (i.e., the temporal relationship between drug exposure and the adverse event onset/course is unreasonable or incompatible, or a causal relationship to study drug is implausible).

The severity of each adverse event must be recorded as one of the choices on the following scale:
MILD No limitation of usual activities
MODERATE Some limitation of usual activities
SEVERE Inability to carry out usual activities

The expectedness of an AE must be indicated when reporting adverse events. An unexpected adverse event is any adverse experience for which the specificity or severity of the event is not consistent with the current labeling.

A serious adverse drug event (SAE) is defined as any adverse event that occurs during the study that results in any of the following outcomes: death, a life-threatening adverse event (i.e., the participant was at immediate risk of death from the event as it occurred; does not include an event, that had it occurred in a more severe form, might have caused death), inpatient hospitalization or prolongation of existing hospitalization (hospitalizations scheduled before enrollment for an elective procedure or treatment of a pre-existing condition which has not worsened during participation in the study will not be considered a serious adverse event), a persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions), a congenital anomaly/birth defect, a medically important event or required medical intervention to avoid one of the above outcomes. In addition to the above procedures for AEs, all SAEs will be reported to the IRB within 24 hours of recording. All serious adverse event information will be followed until resolution or an appropriate end point is reached. This may involve contacting other clinicians responsible for the participant's care to obtain information on diagnoses, investigations performed and treatment given

Fatal or life-threatening, unexpected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the Sponsor-investigator. Serious, unexpected adverse events that are not fatal or life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor-investigator.

14. Privacy, Data Storage & Confidentiality –
Data stored is password protected and kept in a special computer volume to which only our (a) network administrator and (b) senior data manager have access rights. Participant information on the computer files is identified only by a study-specific identification number. Only approved research

staff has access to the data. All faculty and staff have taken and passed Duke's required training modules on HIPAA regulations for protecting participants' privacy, and the Center for Developmental Epidemiology, where the study records are held, observes all approved security rules to protect the data. (3) Protocols and tapes will be kept in locked file cabinets in a locked storage room in a research facility that is locked at night. (4) Written reports and papers will not identify subjects or make identification possible, without the written consent of the person concerned. Our data storage plans have been developed to be fully compliant with HIPPA safeguards. Electronic communication between the research team and participants will follow Duke Medicine Electronic Communication policy guidelines, including encryption of Sensitive Electronic information (SEI) by prepending the Subject line of the message with the string:
(secure).

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