INSTITUTIONAL REVIEW BOARD
SUMMARY
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STUDY TITLE: Interstitial Cystitis – Examination of the Central Autonomic Network (ICECAN)
NCT03008382

A. PURPOSE OF THE STUDY

To determine whether abnormal Autonomic Nervous System (ANS) activity in subjects with Interstitial Cystitis / Bladder Pain Syndrome (IC/BPS) causes or results from their Chronic Pelvic Pain (CPP) state, and to elucidate underlying brain mechanisms.

B. HYPOTHESIS / SPECIFIC AIMS

Our previous grant cycle (ICEPAC) found that high frequency heart rate variability (hfHRV), a measure of parasympathetic function, and baroreflex sensitivity (BRS), a marker of ANS flexibility, were decreased in subjects with IC/BPS in comparison to healthy control subjects, and most significantly, in comparison to subjects with another type of CPP called myofascial pelvic pain (MPP). ***These findings underscored the importance of carefully phenotyping subjects for the presence of each CPP type.*** We therefore believe ANS activity may control endogenous pain modulation and modulate the IC/BPS disease state. The proposed longitudinal set of studies is designed to address this question, deepen our pathophysiologic understanding of CPP in general, and lead to new treatment insights tailored to this population.

The findings from the last cycle, coupled with literature referenced in the background below, particularly related to periaqueductal gray (PAG) – prefrontal cortex (PFC) connections in irritable bowel syndrome (IBS) compared to ulcerative colitis (UC), and in subjects with CPP due to endometriosis, led us to **hypothesize that the ventromedial (vm) PFC normally biases the ventrolateral PAG to inhibit ascending nociceptive pathways and urgency information from the bladder. We believe loss of this vmPFC bias is both necessary and sufficient to incite an IC flare in a subject with IC/BPS and that abnormal ANS activity reflects this reset switch in the vlPAG.**

Abbreviations
ACP: active change in posture
ANS: Autonomic Nervous System
ANS-R: ANS Responsiveness
BP: blood pressure
BRS: baroreflex sensitivity
CPM: central pain modulation
CPP: chronic pelvic pain
DTI: diffusor tensor imaging
EPI: echoplanar imaging
fc: functional connectivity
fMRI: functional magnetic resonance imaging
IC/BPS: interstitial cystitis/ bladder pain syndrome
IBS: irritable bowel syndrome
HC: healthy control
HR: heart rate
HRV: heart rate variability (hf: high frequency; (v)lf: (very) low frequency)
MI: myocardial infarction
MPP: myofascial pelvic pain
PAG: periaqueductal gray
PFC: prefrontal cortex (vm: ventromedial; dl: dorsolateral)
TP: total power (referring to all frequencies of HRV)
VBM: voxel-based morphometry

Specific Aim 1 – Do changes in HRV precede or follow changes in IC/BPS disease activity? In a cohort of CPP subjects, determine if hfHRV and BRS (1a) change before or after changes in CPP disease activity monitored weekly; (1b) at baseline predict clinical status at 4, 12, 16 and 24 weeks after enrollment, irrespective of treatment; ***Hypothesis* and Data Interpretation***: If HRV abnormalities set the stage for IC/BPS, HRV index changes will precede changes in the clinical and state in IC/BPS more so than in MPP.* In contrast, improvement in HRV/BRS following symptom change will suggest the opposite, and no change in HRV/BRS associated with clinical change or will imply an unrelated "bystander" effect.

Specific Aim 2 – Does modulating HRV influence CPP disease activity? Determine the effect of directly modulating hfHRV using beta-blocker or placebo, in a 24-week crossover design study. ***Hypothesis* and Data Interpretation***: If improving hfHRV truly engenders a positive outcome and is not just a consequence of better health, increased HRV will decrease disease activity and improve pain modulatory state in IC/BPS more so than in MPP.* No effect will suggest that HRV changes result from, rather than produce clinical benefit.

Specific Aim 3 – What fundamental reprogramming underlies changes in HRV and in clinical state?

Mechanistic: Determine if HRV changes parallel improved connectivity between the vmPFC and the PAG or

increased **PAG size with connectivity and morphometry data. Independent variables of increased PAG size and vmPFC-PAG connectivity will be regressed against HRV change, clinical improvement, and pain modulatory state.**

Hypothesis* and Data Interpretation*: If higher HRV derives from improved PFC-PAG connections, hfHRV rise should parallel tightened PFC-PAG connectivity and hypertrophy of the PAG. The absence of a relationship will suggest that improved clinical status will be the sole determinant of these brain changes.

C. BACKGROUND, SIGNIFICANCE, AND RATIONALE

The European Association of Urology defines chronic pelvic pain (CPP) as pain unrelated to a structural process, perceived in structures related to the pelvis recurrent for at least 6 months and often associated with negative cognitive, behavioral, sexual, and emotional consequences [1]. A 1996 study [2] estimated that 9 million women in the US between ages 18 and 50, some 15% of the female population at the time, suffered from CPP. A 1994 Gallup poll estimated direct costs of CPP at nearly $1.4 billion in 2013 dollars, with 15% of women reporting lost work revenue and 45% decreased work productivity [3].

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) afflicts 3 to 8 million women in the US [4]. Symptoms of IC/BPS reduce quality of life, suppressing both social well-being and physical function[5]. The chronic pain, voiding dysfunction, sleep deprivation and associated co-morbid conditions interfere with relationships and employment [6, 7] with significant direct (doctor visits, medication, surgery) and indirect (loss of productivity) economic impact, currently exceeding $100 million per year [8].

Despite 25 years of NIH funded investigation of CPP focused on the end-organ, no new therapies have emerged, and the pathophysiology remains obscure. Recent studies emphasize a key role for central processing networks [9]. In our first cycle of funding, we moved from the more common focus on central pain processing to study autonomic outflow. We hypothesized that IC/BPS would feature abnormalities in autonomic function not seen in other CPP disorders such as MPP (myofascial pelvic pain) so that these changes could not simply be construed as due to CPP, since they occur in IC/BPS but *not* in other types of CPP. This hypothesis found support in the results of the prior cycle (see Section C, progress report). These findings underscore the critical importance of a careful phenotype in CPP subjects, because the comparison of CPP subtypes would clearly have been impossible without it. *Since such phenotyping rarely occurs in routine clinical or scientific practice, we emphasize its significance as a novel part of our approach to CPP, and as a major theme in the current proposal.*

Significantly, our ICEPAC studies showed decreased ANS responsiveness (ANS-R) in subjects with IC/BPS; both in vagal tone, measured by high-frequency heart rate variability (hfHRV), and in sympathetic reactivity, measured by baroreflex sensitivity (BRS), both at rest and during orthostatic challenge. While decreased vagal tone occurs in other chronic pain disorders like fibromyalgia [10, 11], chronic neck and shoulder pain [12], chronic back pain [13] and irritable bowel syndrome [14], what is noteworthy is that two CPP disorders, IC/BPS and MPP differed in their overall ANS-R. *Until now, low vagal tone was viewed as a general "marker" of chronic pain. Our findings suggest a new, disease-specific interpretation, perhaps reflecting ANS-R as a marker of specific cortical-brainstem networks that influence central modulation of pain experiences.*

This proposal aims to move the science of CPP from simple associations towards an investigation of cause and effect relationships. We will determine whether the striking changes in ANS-R contribute meaningfully to the pathogenesis of IC/BPS through 3 aims:

1) Careful longitudinal repeated measures in individual subjects to determine if ANS-R changes precede clinical changes;
2) Assessing the impact of an intervention designed to change ANS-R on the clinical course of IC/BPS;
3) Evaluating changes in brain connectivity between the prefrontal cortex (PFC) and the periaqueductal gray (PAG) associated with changes in ANS-R and improved disease status.

In the first aim, we will also study ANS-R to a noxious stimulus, reported as deficient in subjects with IC/BPS [15]. In that report, the authors did not phenotypically separate IC/BPS from MPP by evaluating pelvic tender points; therefore some of these subjects may have also had MPP [15]. Despite this limitation, their

findings support the hypothesis of an association between IC/BPS and central autonomic and perhaps pain dysregulation. In aim 1, we expect that changes in ANS-R will predict and precede clinical change in IC/BPS, and less so in MPP.

Aim 2 will modulate ANS-R with a beta-blocker or placebo using a crossover design and examine subject response to continued standard therapy. Beta-blocker treatment improves ANS-R measures. For example atenolol increased lfHRV by 45%, hfHRV by 84% and total power (TP) by 68% in healthy adults [16], decreasing lf/hf power by 36%. In a large epidemiological study [17], subjects on beta-blockers increased TP by 13%, hfHRV by 22%, and very low frequency (vlfHRV) by 25%, but with a 12% decrease in lf/hf power. In decompensated heart failure, which comes with high sympathetic stress, beta-blocker treatment resulted in 41% higher hfHRV [18]. After acute MI, beta-blockers increase hfHRV and reduce lfHRV [19], and improve parasympathetic tone recovery, which correlates with improved outcome, and decrease morning sympathetic predominance [20]. Subjects randomized to propranolol had a greater increase in hfHRV power at 6-weeks and after 21 months of follow up. In coronary artery disease subjects [21], hfHRV power increased by 64% after atenolol and by 62% after metoprolol for 2 weeks. We expect the salutary effects of beta-blockade to manifest as better response to standard treatment in subjects with IC/BPS compared to MPP.

Central sensitization, as seen in decreased pain tolerance, increased bladder sensitivity and hypervigilance, occurs in subjects with IC/BPS [22, 23]. These observations have not been studied by fMRI or DTI. The PFC–PAG pathway is key to pain-inhibiting circuitry [24], and vmPFC connects tightly to PAG [25], and facilitates disease relevant pain only, implying that cortical settings can allow or disallow ascending signals leading to a pain experience [26]. In IBS, reduced lateral PFC influence on PAG appears to permit ascending nociceptive signals access to higher centers [27]. Consistent with this role, stimulating the lPFC-PAG pathway by transcranial magnetic stimulation [28] inhibits pain and the lPFC atrophies in the setting of chronic pain [29]. *Of critical interest to this application, the vmPFC is not only anatomically linked to the PAG [25], and critical to pain regulation, it is also the cortical structure that is most closely linked to the brainstem autonomic networks that are reflected by ANS-R.*

Indeed, our studies [30] suggest that higher ANS-R indicates a greater capacity for context and goals to modulate emotion, closely linked to the output of vmPFC. Roy et al. [31] conceptualize the vmPFC as a "system of systems" linking conceptualization, context, emotion and response. Thus, its connection to the PAG may bias more caudal brainstem networks to specific modes that reflect particular behavior patterns and their autonomic accompaniments. ANS-R may be the "poor man's" marker of vmPFC outflow to the PAG. *Relevant to this proposal, vlPAG has just the right connections and functions to modulate flares and remissions in IC/BPS.* It (a) receives input from the PFC, (b) modulates ascending C-fiber visceral nociceptive input [32], (c) integrates autonomic and nociceptive responses [33], (d) increases hfHRV, an indicator of parasympathetic activity, in proportion to chronic pain relief produced by deep brain stimulation [34], (e) is activated during voiding and micturition and triggers micturition [35-38] and (f) takes part in REM/non-REM switching during sleep, an area of significant dysfunction in most of our subjects with CPP.

D. DESIGN AND METHODS

This multi-site trial will recruit 3 groups of female subjects ages 18-80 years: 1) IC/BPS (n=60); 2) MPP with or without IC/BPS n=60); 3) 60 HCs [only for fMRI] (18 per group for MRI). Recruitment will occur from the prior ICEPAC cohort from Dr. Tu's K award cohort at Northshore, and from specialty clinics associated with MCW and NorthShore University HealthSystem (Chicago). We expect to enroll 50 participants from each group at MCW/FMLH. We expect a 15% dropout over the 24 weeks, so that we expect 60 starters and 51 completers in each group. Please see Table 1 for a list of study activities and responsibilities for each study site.

Table 1. Site coordination, procedures and recruitment			
	FMLH	Case	Northshore
Coordination	Responsibility for all activities, fMRI, HRV integrity, psychiatric red flags and data	Psychiatric red flags and data	Diagnostic accuracy
Procedures	Enroll, HRV, Home HRV (ACP), BRS, Computational model, analyze psychological data	Analyze psychological data	Enroll, on-site HRV (ACP)
Peak Recruitment Rate (Total n at each site)	30 per year with hope to collect complete from 79 total participants	Case has recruited 13 total but will no longer be recruiting and will only be analyzing data.	12 per year, 46 total

Overall Pelvic Pain Subject Inclusion & Exclusion criteria

General inclusion criteria for subjects will include:

- Women aged between 18 and 80 years old.
- Healthy controls and subjects diagnosed with IC/BPS or MPP.
- For IC/BPS - ≥3 months chronic pelvic pain, pressure or discomfort perceived to be related to the urinary bladder accompanied by at least one other urinary symptom like persistent urge to void or frequency. Confusable diseases as the cause of the symptoms must be excluded, particularly recurrent urinary tract infection (UTI).
- Women with MPP - ≥3 months of non-cyclic continuous pelvic pain unrelated to bladder state and a minimum of 2 of 5 examined pelvic floor TPs scoring at least 4 out of 10 on a numeric rating scale using 2 kg pressure applied with the index finger.
- Provision of informed consent prior to any study specific procedures.

General exclusion criteria for all subjects will include:

- Known nervous system conditions including but not limited to diabetic neuropathy, Parkinson's disease, Alzheimer's disease, multiple sclerosis, strokes, seizures, etc.
- Baseline heart rate < 50 bpm; blood pressure ≥140/80 mmHg at rest or uncontrolled hypertension; or hypertension requiring more than two drugs for control.
- Pregnant, attempting to become pregnant, or breast-feeding.
- Unevaluated hematuria or infection at the time of enrollment.
- Pelvic or bladder neoplasm or infection.
- Severe and uncontrolled asthma, inflammatory arthritis, connective tissue or auto-immune disorders.
- Evidence of unstable medical disorder, such as kidney (rising creatinine, or end-stage renal failure) or liver impairment (rising AST or ALT, or end-stage with coagulopathy), poorly controlled significant cardiovascular (CHF), respiratory, endocrine (diabetes – A1c > 9 – or untreated thyroid dysfunction) or uncontrolled psychiatric illness (such as untreated depression, psychosis, etc.).
- Treatment with a drug or medical device within the previous 30 days that has not received regulatory approval.
- Use of hormones (except thyroid replacement or sex hormones. These may include the following birth control methods- IUD based, periodic injections, oral, patch, ring, and implant (3-year duration) which are acceptable). Hormone replacement therapy, such as growth hormone, is not acceptable.
- Current, ongoing substance or alcohol abuse.
- Current use of 150 mg or more of narcotics or morphine equivalent (or inconsistent dosages or frequency – varying by > 50 mg morphine equivalent per day).

- Previous augmentation cystoplasty, cystectomy, cytolysis, or neurectomy.
- General anesthesia for a non-traumatic procedure (e.g. colonoscopy, cystoscopy etc.) or minor procedure such as skin tag removal etc. in the past 2 weeks
- A traumatic procedure that does NOT involve the pelvic floor (e.g. shoulder surgery, cardio-pulmonary surgery) in the past 4 weeks
- A traumatic procedure that DOES involve the pelvic region (e.g. hysterectomy, hip surgery etc.) in the past 6 weeks
- Current use or planned use of anticholinergic medications.
- Current use or planned use of beta-agonists.
- Unwillingness to take a beta-blocker and placebo, or planned use of beta-blocker(s) other than the study medication.
- Previous allergic or serious reaction to beta-blockers.
- Current use or planned use of certain prohibited medications-**See Restricted and Prohibited Medications in Appendix 1**
- Initiation of neural stimulator in the last 30 days.
- Any on-going or pending medical, health or disability related litigation, or current pursuit of disability.
- Any condition that in the judgment of the investigator and the internal advisory panel would interfere with the subject's ability to provide informed consent, comply with study instructions, place the subject at increased risk, or which would clearly confound the interpretation of the study results (specific reason will be documented).
- Current participation in another clinical trial that interferes with ICECAN policies and procedures.
- Investigators, study staff and their immediate families.
- Inabilities to speak, read, and understand English.
- Known allergy to adhesives.
- Initiation of any new treatment class in the last 30 days, or intent to initiate a new class of treatment in the study. Treatment classes include:
    - Pelvic injection
    - Pelvic floor therapy
    - Agents with specific FDA approval for IC/BPS or MPP (e.g., Elmiron)
    - Anticonvulsants
    - Tricyclic agents
    - Intravesical therapy or Botox
    - Bladder hydrodistention

Short term use of albuterol, levalbuterol, or pirbuterol is allowed while subjects are actively receiving metoprolol/ placebo for a maximum of 7 days duration. If the acute illness requiring a short-acting beta agonist has not resolved in 7 days, metoprolol/ placebo can be held for up to 14 days. After 14 days, metoprolol/ placebo can be resumed and the remainder of the 8-week treatment course may be completed. Contact the PI if short-acting beta agonist use is required for more than 21 days.

<u>Healthy controls:</u>
Healthy female subjects will be recruited by nomination by patients, screening from the BANK Medical College of Wisconsin fMRI Research Program, flyers and advertisement. Only members of the ICECAN research team at MCW will have access to the fMRI Research bank. A history and physical will be obtained by a physician and controls will be required to have no history, symptoms or signs of all the exclusion criteria described above plus:

- Chronic fatigue syndrome, Raynaud's syndrome, Rheumatoid arthritis, Fibromyalgia, Temporo mandibular joint disorder, Dysmenorrhea, Endometriosis, Dyspareunia, Functional gastrointestinal disorders including Cyclic vomiting syndrome, Irritable bowel syndrome, Functional abdominal pain syndrome, Functional dyspepsia; Chronic idiopathic nausea, Migraine headaches, Abdominal migraine, Syncopal migraine; Reflex syncope, Postural tachycardia syndrome, Orthostatic intolerance syndrome,

Post-traumatic stress disorder, Panic disorder, Generalized anxiety disorder, Adjustment disorder with depressed mood, Major affective disorder, Complex regional pain syndrome, Diabetes, Glucose intolerance, Currently active asthma, Multiple chemical sensitivity, and Periodic limb movements in sleep. Diagnosis of IC/PBS, MPP or chronic pelvic discomfort or chronic pain disorder of any type that consistently interferes with life's functions at work or home > 2 days/week for more than one month.

Protocol and Procedures

All IC/BPS and MPP subjects will participate in both **Aim 1 and Aim 2**. In contrast, aim 3 is an optional sub-study and will only include 18 subjects from each group.

All activities are identical for all sites except the breathing test (Valsalva maneuver is only conducted at MCW). See the Data Collection Schedule in Appendix 3.

Aim 1 and Aim 2 – Experimental Group

Baseline Visit: Week 0, Visit 1

Recruitment will occur from the prior ICEPAC cohort from Dr. Tu's K award cohort at Northshore, and from specialty clinics associated with MCW and NorthShore University Medical Group (Chicago). Up to 120 IC/BPS and MPP subjects (60 per each group) will be recruited study wide. We expect to enroll up to 100 IC/BPS and MPP subjects (50 per group) from Froedtert Memorial Lutheran Hospital (FMLH). FMLH subjects will be recruited from the OB/GYN and Neurology Clinics. A member of the research team at FMLH introduces subjects to this study. Consent is obtained by a member of the research team. Once the subject has had adequate time to consider participation, understands all of the risks and benefits and would like to participate, the subject will sign informed consent and HIPAA documents.

Subjects will be pre-screened in person or on the phone. An in-person pre-screen takes place in the MCW Neurology Research Rooms at FMLH. If the subject is able to participate, subject will sign the informed consent form before completing a baseline evaluation. The baseline evaluation occurs either following consent or at another date convenient for them. Subjects must complete all baseline activities within 2 weeks, or before their Week 4/Visit 2. The baseline evaluation is comprised of a general examination[39], a set of questionnaires (Appendix 3), background history documenting date of diagnosis, medications tried, duration of each treatment and dosing, surgeries and therapeutic and exploratory procedures performed (Appendix 3). A pelvic examination will be performed with Chronic Pelvic Pain subjects only. HRV/BPS (See Active Change in Posture – ACP below) tests is administered to the subject (see procedures for details). Subjects will complete the uroflow measurement and Valsalva maneuver in the MCW Neurology Research Rooms with a member of the research team present.

Weekly Home Checks will occur for 24 weeks following consent. Once a week, subjects will complete a 24-hour HR recording, voiding diary and the ACP recording using the eMotion Faros 360° portable EKG device. A member of the research team will contact the subject via phone, text, email or skype each week to remind them to complete the nighttime ACP, followed by the 24-hour HRV recording, voiding diary and the weekly questionnaires via the EMA App and link to RedCap (or via paper). This will ensure compliancy and will give the subject the opportunity to ask any questions. The researcher will contact them via phone or via skype to monitor the subject while the ACP recording is completed from home just prior to the subject's bedtime for the first few recordings until the subject is comfortable completing the recording by herself. The HR recording will be uploaded to MCW's secure server at the subject's next hospital visit. Throughout the study, subjects flare activity will be monitored via phone calls, texts or emails and recording flares in the EMA application (Appendix 2) on the smartphone. This will be a Daily Flare Question in the EMA which is a set alarmed time to sound as a reminder for the duration of the subject's participation.

The following weeks after each site visit and the 24 hours before visit 5, subjects will complete the final ACP recording prior to bedtime and 24-hr HRV recording to ensure comparability of the HRV recording at home to the one performed at the matching on-site visit. Subjects will also complete the 24-hr voiding diary, a set of

questions using the ICECAN mobile App installed on a pre-loaded smartphone and questionnaires via a Redcap link. The recorded HR data will be downloaded from the monitor and standard HRV analysis for steady state (supine rest phase of ACP test) and time-varying HRV analysis will be performed, as described above, extending over the 24-hr period, as we have shown [40]. Deidentified 24-hour HRV and ACP recordings will be sent to Dr. Julian Thayer's research group at University of California Irvine for analysis.

Follow-up Visits: Weeks 4, 12, 16, 24, Visits 2-5
Subjects will arrive to MCW Neurology Research Rooms to repeat the following: review of comorbidities, tender points exam, questionnaires, and HRV/BPS (ACP) tests. Subjects will complete the Valsalva maneuver in the MCW Neurology Research Rooms with a member of the research team present. Chronic Pelvic Pain subjects will complete a repeat pelvic examination at Weeks 12 and 24.

MPP and IC/BPS subjects will be randomized to receive 8 weeks of either placebo (a pill with no active agent), or metoprolol (a pill that reduces the impact of the brain's "fight or flight" circuits) using Piantadosi's Randomization software [41], overseen by Dr. Pippa Simpson in Biostatistics. Metoprolol is in the class of "beta-blockers" commonly used for mild blood pressure control, and also commonly used for migraine. Subjects will be administered 8 weeks of metoprolol or placebo starting at their Week 4 Visit. Subjects will complete a 4-week washout period (Week 12-16) and will be administered 8 weeks of crossover (Weeks 16-24).

If for some reason it is not possible for the patient to collect the study product while on-site during a study visit, the study drug may be mailed to the patient if the site pharmacy agrees to ship the study medication to their study participant. This will ensure the study team is able to provide the study product to subjects if the manufacturer is unable to refill the prescription prior to the site's supply running out. The research pharmacy in Froedtert has agreed to ship the study medication to the subject(s) via FedEx. The study team will provide the research pharmacy with the FedEx Airbill and will record the tracking number on the airbill to track the date and time of the delivery to the participant(s). Participants will then return the study medication at the next study visit. If for some reason the study participant(s) are unable to return the study medication to the site, they will be sent a FedEx airbill and supplies to use to return the study medication to the site. The study team will then record the tracking number of the package to track its return. The study team will also inform the participants to place the medication in the FedEx packaging, place the airbill on the packaging and drop it off at any FedEx location.

Optional: Blood will be drawn (~ 50 mL, a little more than 3 tablespoons) at each in-person visit at weeks 0, 4, 12, 16, and 24 for chronic pelvic pain subjects (healthy control only at Baseline and Final visits; 2 draws total). We will determine (1) if immune or inflammatory factors play a role in pain cycles and (2) if energy production in cells might be impaired in chronic pelvic pain. A portion of the blood plasma/serum will be sent to Dr. Lori Birder's laboratory at the University of Pittsburgh for additional related analysis. The sample will be labeled by a number and will not contain any information that can be used to directly identify subject. This portion of the study is critical to gather new information about pelvic pain, which is very poorly understood. We highly encourage subjects to participate in this portion of the study. However, given how difficult it can be to obtain blood from some people with IC/BPS, we do allow subjects to opt out of the blood draw portion of this study and still participate in the other parts.

Urine will also be collected (~ 50ml) at each in person visit at weeks 0, 4, 12, 16 and 24 for chronic pelvic pain subjects (healthy control only at Baseline and Final visits). Samples will be sent to Dr. Lori Birder for related analysis.

If for some unforeseen reason the physician assessment (MEDYSA) is unable to be completed during any of the study visits, the study investigator may complete this with the study participant over the phone.

Every subject will receive a general wellbeing follow-up phone call or email 4 weeks after their study completion.

Healthy Controls
50 healthy control subjects will be enrolled at FMLH and will also complete this study in 24 weeks that include 4 long site visits and weekly home check visits. Subjects will complete the weekly home check visits during their first 3 weeks in the study and then again after week 12 or 20 for an additional 3 weeks.

During the first long visit they will complete a general exam and physician evaluation, HRV/BRS (ACP), Valsalva maneuver (MCW only), and questionnaires (Appendix 3)).

Subjects will complete a 24-hour HRV/BRS recording from home once a week for 6 weeks. A member of the research team will contact the subject each week while they complete the weekly questionnaires and ACP recording until they are comfortable and understand this process. This will ensure compliance and will give the subject the opportunity to ask any questions. The researcher will remain on the phone or via skype to monitor the subject while the ACP recording is completed from home just prior to the subject's bedtime for the first few recordings until they are comfortable completing this on their own.

Subjects will return to the hospital 3 weeks later to complete the second long site visit procedures including a physician evaluation, HRV/BRS (ACP), Valsalva maneuver, and questionnaires (Appendix 3).

When subjects return to the hospital for Visit 3, at either week 12 or 20, they will complete the third long site visit procedures including a physician evaluation, HRV/VRS (ACP), Valsalva maneuver, and questionnaires (Appendix 3).

Subjects will complete a 24-hour HRV/BRS (ACP) recording from home once per week for the following 3 weeks. A member of the research team will contact the subject each week to remind them to complete the weekly questionnaires and ACP recording.

Subjects will then return to the hospital for their Final Visit/Visit 4 at either week 16 or week 24 to complete the last study procedures including a physician evaluation, HRV/BPS (ACP), Valsalva maneuver, and questionnaires (Appendix 3).

Every subject will receive a general wellbeing follow-up phone call or email 4 weeks after their study completion.

Aim 1 Observational Alternative Study:

Subjects who meet the study eligibility criteria will be eligible to participate in the Observational Alternative Study. Recruitment will occur from the prior ICEPAC cohort from Dr. Tu's K award cohort at Northshore, and from specialty clinics associated with MCW and NorthShore University Medical Group (Chicago). Up to 20 IC/BPS and MPP subjects from Froedtert Memorial Lutheran Hospital (FMLH) will be recruited to this study from the OB/GYN and Neurology Clinics. A member of the research team at FMLH will introduce subjects to this study. Consent is obtained by a member of the research team. Once the subject has had adequate time to consider participation, understands all of the risks and benefits and would like to participate, she will sign informed consent and HIPAA documents.

If the subject is interested in this research study, she will first complete a pre-screen in person or on the phone to see if she is able to participate. An in-person pre-screen takes place in the MCW Neurology Research Rooms at Froedtert Hospital (FH), Neurology or OBGYN clinics at Moorland Reserve Health Center or Froedtert Main Campus, or at Westbrook Clinic or St. Joseph's Health Center.

If the screening information shows that the subject meets the requirements, then she will be able to start. If the screening information shows that she cannot be in the research, the research doctor will discuss other options with her and/or refer her back to her regular doctor.

If the subject is able to participate, she will complete 3 regular on-site long-visits at weeks 0, 4, and 12. The first long-visit (baseline evaluation) may occur either the same day she provided consent or at another date convenient for her. The baseline evaluation comprises a general and pelvic examination (no speculum), psychological questionnaires, detailed medical interview and a background history questionnaire. This exam may take up to 45 minutes to complete. She will also complete a urine flow measurement, a heart rate recording and a breathing test. The participant will also be asked to provide a urine sample and a blood sample. The blood sample is optional.

Follow-Up Visits Weeks 4 and 12
At each long-visit, the subject will complete a physician exam, a set of questionnaires, provide a 24-hour voiding diary and the following tests: breathing test, heart rate test and a "uroflow measurement," A "uroflow measurement" measures the volume of urine, how long it takes to be released from the body and the speed. The subject will also complete a repeat pelvic examination at Week 12.

The subject will be asked to complete questionnaires at each long-visit including: Hospital Anxiety and Depression Scale (HADS), State-Trait Anxiety Inventory (STAI), Thoughts About Symptoms (CSQ), Pennebaker Inventory of Limbic Languidness (PILL), Multidimensional Pain Inventory (MPI), Female Genitourinary Pain Index (FGUPI), Perceived Stress Scale (PSS), PROMIS Social Roles and Activities, PROMIS Physical Function, PROMIS Fatigue, PROMISE Sleep Disturbance, the Holmes-Rahe Life Stress Inventory, the MAPP II Interactive Brief Pain Inventory, and the Covid-19 Questionnaire. They will complete the Childhood and Recent Trauma Events Scale (RTES) during their first long-visit only.

The subject can skip any question that they do not feel comfortable answering on any of the forms. Although the first questionnaires will be completed in the clinic to keep their answers private, the next will be completed while they are at home. We cannot guarantee that those responses will be kept private and there is a chance that answers may be seen by those who live in or visit their home. If their score is suggestive of emotional problems, study director or a study Co-Investigator and/or Psychologist will provide them with places to help get treatment for these issues within 1 week of their participation. The researchers are required by law to report any abuse or neglect (or suspicion of abuse or neglect) if the subject mentions it to the researchers or if it is suspected.

The 24-hour voiding diary consists of tracking how much liquid comes in and out of the subject's body in a 24-hour period, and how often they void. The subject will be required to measure the amount of everything they drink. The subject will also record the time and amount of each void for the 24-hour period. A special measuring "hat" that fits over the toilet seat will help the subject measure each void. The uroflow measurement requires the subject to urinate on a special toilet in the MCW Neurology Research Rooms at FH. The toilet contains sensors that measure the speed of the urine flow and amount of urine. About one hour prior to this test the subject will be asked to drink a quart of fluid.

The subject will complete a "breathing test" (Valsalva Maneuver) in the MCW Neurology Research Rooms at FH. To complete this test the subject will have wrist straps secured to a table and asked to breathe in deeply and out forcefully through a tube. The subject will have a small band on their arm to measure their heart rate and blood pressure. The subject will complete 4 cycles of breathing: two cycles while lying down flat and two cycles while leaning slightly forward.

The active posture change (ACP) heart rate recording (HRV) will take about 20 minutes to complete. The subject will complete this recording at each long-visit and each week that they are in this study. Heart rate will be recorded with an eMotion Faros 360° portable ECG device. It is small and lightweight, with 3-5 small

adhesive electrode patches placed on the skin of the subject's chest and torso. The subject will lie down flat on a bed and rest for 10 minutes before the recording starts. After that, the subject will lie down for 5 more minutes while their heartbeat is recorded for 5 minutes. The subject will be asked to stand up as quickly as they can and stand still for an additional 5 minutes. The subject may sit down if this test becomes too much for them to complete. Coded 24-hour HRV and ACP recordings will be sent to Dr. Julian Thayer's research group at University of California Irvine for analysis. The subject's sample will be labeled by a number and will not contain any information that can be used to directly identify them.

Blood (optional) will be drawn and urine will be collected (~ 50 mL, a little more than 3 tablespoons) at each in-person visit at weeks 0, 4, and 12. We will determine (1) if immune or inflammatory factors play a role in the subject's pain cycles and (2) if energy production in the subject's cells might be impaired. A portion of the subject's blood plasma/serum and urine will be sent to Dr. Lori Birder's laboratory at the University of Pittsburgh for additional related analysis. Both of the subject's samples will be labeled by a number and will not contain any information that can be used to directly identify them. This portion of the study is critical for us to gather new information about pelvic pain, which is very poorly understood. We highly encourage the subject to participate in this portion of the study. However, given how difficult it can be to obtain blood from some people with IC/BPS, we do allow the subject to opt out of the blood draw portion of this study and still participate in the other parts.

Weekly Home Checks will occur once a week for 12 weeks following consent. Each week, the subject will complete a 24-hour heart rate (HR) recording using the eMotion Faros 360° ECG device, the 24-hr voiding diary, answer a set of questions using the ICECAN mobile App installed on a preloaded smartphone, and complete questionnaires (those mentioned previously) that are sent to the subject's personal e-mail account via a secure Internet connection. The questionnaires will be completed either electronically via REDCap or via paper. The heart rate recording will help us to study how the subject's heart rate changes throughout the day. The subject will place the patches on their chest and torso to match the image found on the instruction sheet provided to them as soon as they get up in the morning. For the first few recordings, a member of the research team will contact the participant, either via telephone, email, skype or text, later in the evening, before the subject goes to bed, to complete an additional recording. The researcher will ask the subject to lie down flat on their bed for 15 minutes. After that, the researcher will instruct the subject to stand up as quickly as they can in front of their bed for another 5 minutes. The subject can sit down if they become too dizzy. The data from the subject's heart rate recording will be uploaded to a secure server by a member of the research team. The researcher will ask whether the subject has had a chance to complete the questionnaires. If in any case the subject is unable to complete the questionnaires online, they will be asked to return paper copies of the questionnaires at their next in-person follow-up visit.

The subject will also complete a Daily Flare Question which asks about flare activity, management and if the flares are affecting them. These Flare Questions will be monitored via phone calls and recording in the EMA application on the smartphone.

After each site visit and the 24 hours prior to visit 3, the subject will repeat the ACP recording prior to bedtime and the 24-hr HR recording, as described above, to compare the HRV recording done at home to the one performed at the matching on-site visit.

If the participants end up not answering some of the EMA questions, we will be asking them the following: At any time during the study, did your pain affect your ability to answer any of the EMA questions? When you didn't answer the EMA questions, what percentage of time was it due to pain? These questions will be asked at each in-clinic visit appointment.

If for some unforeseen reason the physician assessment (MEDYSA) is unable to be completed during any of the study visits, the study investigator may complete this with the study participant over the phone.

Every subject will receive a general wellbeing follow-up phone call or email 4 weeks after their study completion.

Aim 1 -2: Incentives
IC/BPS and MPP subjects will be compensated for their time and travel. Travel will be reimbursed as follows: For travel time between 1 and 2 hours, subjects will be paid $20 per visit and for travel time between 2 and 4 hours, subjects will be paid $40 per visit. They will be compensated for participation a total of $400 by a preloaded credit card for completing at least 80% of the required items for the five major visits at the sites and the at-home monitoring sessions. Subjects will receive $50 after completing all study tasks and Visit 1, $60 after completing all study tasks and Visit 2, $70 after completing all study tasks and Visit 3, $80 after completing all study tasks and Visit 4, $140 after completing all study tasks and Visit 5, and returning the Faros ECG monitor and the pre-loaded Smartphone.

Healthy controls will receive a total of $400 by a preloaded credit card for completing at least 80% of the study activities, returning the Faros EKG, smartphone and completing the 4th and Final visit. This includes the general examination, psychological questionnaires, a breathing test, heart rate test, the uroflow measurement, and at-home participation. They will receive $50 after completing Visit 1/Baseline,$150 after completing all at-home study activities and Visit 2, $100 after returning from the break and completing Visit 3, $100 after completing all at-home study activities, completing Visit 4/Study Completion and returning all study materials.

Observational Alternative Study Subjects will be compensated for their time and travel. Travel will be reimbursed as follows: For travel time between 1 and 2 hours, subjects will be paid at $20 per visit and for travel time between 2 and 4 hours, subjects will be paid $40 per visit. They will be compensated for participation in the study with $250 by a preloaded credit card for completing at least 80% of the required items for the three major visits at the site and the at-home monitoring sessions. Subject will receive $50 after completing all study tasks and Visit 1, $50 after completing all study tasks and Visit 2, and $150 after completing all study tasks, Visit 3, and returning the Faros ECG monitor and the pre-loaded Smartphone.

Subjects may be given the option to redo a study visit if the study data are incomplete or if they need to stop the study drug. If they redo a visit, they will receive payment for the visit which was repeated in the same amount as mentioned above for the corresponding visit.

Subjects who complete the baseline evaluation but are found not to be eligible to participate will be mailed a $25 check.

Aim 3 Optional MRI Sub-study
Subjects who are enrolled in the Experimental Group or Healthy Control Group will be given the option to participate in the *f*MRI sub-study. Chronic Pelvic Pain subjects will be asked to complete 3 fMRI scans that each take roughly 60 minutes to complete. Healthy controls will complete a total of 2 scans: one scan before their Week 4 visit and one scan before their Week 24 visit or final visit. We will enroll 54 subjects able to commute to MCW, 18 IC/BPS, 18 MPP (none with both diagnoses) and 18 HCs. The Safety Screening Questionnaire is completed prior to the scan and is verified by the *f*MRI technician. If it is determined that the subject is safe to complete the scan, the technician will introduce them to the scanning procedure. The MRI Experience Questionnaire (MEQ) is completed after the MRI procedure to assess the subjects experience during the MRI.

Sub-study Inclusion and exclusion criteria:

- Between 18 and 80 years old.
- Must be female.
- Must speak, read, and understand English.

- Must be right-handed.
- Does not have orthodontic braces or permanent retainers.
- Must pass the standard MR safety screening as required by the MR Safety Committee. For example, subjects with aneurysm clips, pacemakers, implanted stimulators, and some types of prostheses will be excluded.
- Active cyclic or chronic vomiting will be excluded.
- Subjects who have phonophobia will be tested to see if the noise produced by the MRI is tolerated. Subjects who are unable to tolerate this noise will be excluded from the study.
- Must be able to remain in the scanner for up to 1 hour without moving.
- Carries a diagnosis of chronic pelvic pain x 3 months or more, either interstitial cystitis or myofascial pelvic pain.
- Must not be pregnant or plan to become pregnant in the next 6 months.
- No evidence of any neurologic disorder such as Parkinson's disease, Diabetic Neuropathy, etc.
- No subjects with chronic dizziness.

MRI Sub-study Protocol and Procedures

18 subjects from each sub-group will be approached, screen and consented to undergo an fMRI scan. A3T machine will be utilized for these studies. They will undergo fMRI for connectivity, DTI and VBM at weeks 0 (or within the 4 weeks prior to visit 2), 12 and 24 (inverted triangles Figure 1) with the regular major visit battery (Table 2). Each subject is required to complete a urine pregnancy test prior to the scan unless they are no longer menstruating.

Aim 3: Incentives

IC/BPS and MPP subjects who complete the sub-study will be compensated for their time and travel following each scan. Subjects will receive $75 by a preloaded credit card after completing each of the 3 fMRI scans.

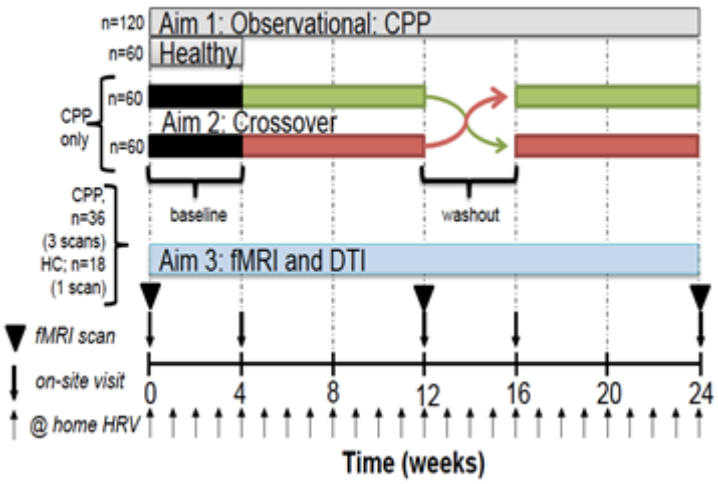
Healthy controls will receive $75 by a preloaded credit card following the completion the first fMRI scan and $75 after each subsequent fMRI scan.

E. TOTAL NUMBER OF HUMAN RESEARCH SUBJECTS PROPOSED FOR THIS STUDY AT THIS SITE AND GLOBALLY. WHAT ARE THESE NUMBERS BASED ON?

At FH/MCW, we will enroll 3 groups of female subjects ages 18-80 years: 1) IC/BPS (n=50); 2) MPP with or without IC/BPS n=50); 3) HCs only (n=50) for fMRI (18 per group for MRI). All subjects will participate in both Aim 1 and Aim 2. Recruitment will occur from the prior ICEPAC cohort from Dr. Tu's K award cohort at Northshore, and from specialty clinics associated with MCW and NorthShore University HealthSystem (Chicago). We expect a 15% dropout over the 24 weeks, so that we will have 60 starters and 51 completers in each group. Please see Human subject section for inclusion and exclusion criteria.

We will enroll a total of 54 subjects to the fMRI sub-study who are able to commute to the Medical College of Wisconsin (MCW), 18 IC/BPS, 18 MPP (none with both diagnoses) and 18 HCs with the goal of completing all 3 scans in 12 subjects per CPP group and two scans in HC's. CPP subjects will undergo fMRI for connectivity, DTI, VBM, and spectroscopy of the PAG at weeks 0, 12 and 24 (inverted triangles Figure 1) with the regular major visit battery (Table 2).

F. DRUGS OR PROCEDURES



		Visit 1		Visit 2	Visit 3	Visit 4	Visit 5
	Week	0		4	12	16	24
	Duration	3h		3h	3h	3h	3h
General exam		X					
Physician exam		X		X	X	X	X
Questionnaires		X		X	X	X	X
Heart rate test		X		X	X	X	X
Breathing test		X		X	X	X	X
Pelvic exam		X			X		X[1]
Home Checks	Done weekly for 24 weeks (40 min)						

X[1] Subjects in the experimental group only.

Figure 1 and Table 2 summarize overall study design. (Note that Aim 1 Observational Study component applies to all subjects and should not be confused with the Observational Alternative Study)

Psychological Scale Abbreviations
BPI : Brief Pain Inventory
HADS : Hospital Anxiety and Depression Scale
RTES: Childhood and Recent Trauma Event Scale
FGUPI: Female Genitourinary Pain Index
MPI: Multidimensional Pain Inventory
CSQ: Thoughts About Symptoms
PILL: Pennebaker Inventory of Limbic Languidness
PSS: Perceived stress scale
STAI: Spielberger State-Trait Anxiety Inventory
HRLSI: Holmes-Rahe Life Stress Inventory

Physician Examination

Detailed history of comorbidities including chronic fatigue syndrome, temporomandibular joint disorder, Raynaud's, fibromyalgia, asthma, rheumatoid arthritis, syncope, postural tachycardia syndrome, chronic idiopathic nausea, functional dyspepsia, functional abdominal pain, IBS, migraine, syncopal migraines, panic disorders, PTSD, a screening questionnaire that will be completed by subjects and reviewed by the research coordinator. Positive answers are followed up by the physician-investigator to determine if the subject has the disorder (ranked as definite, probable, possible, absent). Severity is quantified on an 11-point disease activity numeric rating scale (NRS) for comparison with CPP disease activity. The PROMIS Sleep Disturbance will help quantify sleep effectiveness. Chronic pelvic pain subjects will also have a pelvic examination completed at time of consent, week 12 and week 24.

Questionnaires

1. The Childhood and Recent Trauma Events Scale (RTES) includes 7 questions and will determine the independence of childhood trauma from adult trauma as a factor associated with IC/BPS. Questionnaires may be completed under the direct or indirect (via video link) supervision of a research coordinator to enhance adherence.

2. Depressive symptomatology will be assessed with the Hospital Anxiety and Depression Scale (HADS). Adults rate each of the 14 items on a four-point Likert scale indicating how frequently they experience each symptom.

3. Symptoms of anxiety will be assessed with the State-Trait Anxiety Inventory (STAI)[42]. The STAI is a self-report measure developed to help distinguish between a person's state anxiety and their trait anxiety. Subjects complete 40 items on a four-point likert scale indicating how frequently they experience each symptom.

4. Cognitive patterns of thinking about pain will be measured with the Thoughts About Symptoms (CSQ). The CSQ is a self-report measure developed for adults with chronic pain conditions to assess their negative thinking patterns regarding pain. Adults will complete 8 items on a seven-point likert scale indicating the degree to which they catastrophize about different aspects of their pain.

5. Nervousness, distressed and unhappy physical symptoms will be measured using the Pennebaker Inventory of Limbic Languidness (PILL) Adults will rate a 54-question self-test (http://counsellingresource.com/lib/quizzes/misc-tests/pill/) [43] to quantify somatization.

6. The Multidimensional Pain Inventory (MPI) is a self-report instrument composed of 61 items that form 13 scales assessing cognitive, behavioral and affective aspects of pain as well as three scales that measure impact on function. It has been rigorously examined for psychometric properties[44].

7. The Female Genitourinary Pain Index (FGUPI) is a valid and reliable condition-specific instrument that can be used to quantify symptoms in men and women with bladder/urologic pain. This questionnaire also discriminates between subjects with IC/BPS and other diagnoses. Clemens et al. demonstrated that the score in the GUPI and the scores in the Interstitial Cystitis Problem Index and Interstitial Cystitis Symptom Index correlated highly [45]. This questionnaire consists of 9 questions regarding location of pain, pain associated with certain activities frequency of urination, sensation of not emptying the bladder and how the symptoms affected daily activity [46]. This questionnaire has been chosen since it is currently used by the NIH-MAPP group and will allow for comparison of results.

8. The Perceived Stress Scale (PSS) assesses an individual's perception of stress over the past month [47]. The 10 item questionnaire will ask about thoughts and feelings the respondents have experienced during the last month (http://www.mindgarden.com/documents/PerceivedStressScale.pdf). This scale is designed to measure how subjects find their lives to be overloaded, unpredictable and uncontrollable.

9. The NIH Patient-Reported Outcomes Measurement Information System –PROMIS Social Roles and Activities Short Form and PROMIS Physical Function Short Form ⌈Available at: http://www.nihpromis.org/about/overview (accessed December 30, 2014).] are self-report measures developed for adults with any medical disorder to measure social and physical these functioning. Subjects rank each of the 16 items on five-point Likert scales indicating how much difficulty they have doing common activities.

10. The 8-item version of the PROMIS Sleep Disturbance will help quantify sleep effectiveness.

11. The PROMIS Fatigue short form assesses fatigue over the past seven days. It evaluates a range of self-reported symptoms, from mild subjective feelings of tiredness to an overwhelming, debilitating, and sustained sense of exhaustion that likely decreases one's ability to execute daily activities and function normally in family or social roles.

12. The Holmes-Rahe Life Stress Inventory (HRLSI) is a self-report instrument composed of 43 life events. Subjects are instructed to mark each life event that they have experienced in the previous month or two months.

13. The MAPP II Interactive Brief Pain Inventory (BPI) will be used to conduct brief pain inventory at each in-person visit for pain intensity experienced by the subject in the past 7 days before their visit.

14. If the participants end up not answering some of the EMA questions, we will be asking them the following: At any time during the study, did your pain affect your ability to answer any of the EMA

questions? When you didn't answer the EMA questions, what percentage of time was it due to pain? These questions will be asked at each in-clinic visit appointment if some questions were previously missed.

15. The Covid-19 questionnaire will be administered at each in-person visit and involves how Covid-19 has affected the participant emotionally.

Physiologic Studies Methodology

During the following battery of tests (Active Change of Posture, and Valsalva Maneuver), a surface ECG monitor (eMotion Faros 360°, Mega Electronics Ltd, Finland) and a noninvasive continuous hemodynamic monitoring system using the plethysmographic pulse pressure waveform from a finger cuff (Nexfin, Edwards Lifesciences Corp, Irvine, CA) will be attached to the subject. The Nexfin will only be used at the MCW site due to availability of equipment and expertise in the field. These devices will continuously record ECG and blood pressure waveforms and derived RR-intervals (heart rate) and beat-to-beat systolic, diastolic and mean arterial blood pressure [48, 49]. BRS parameters will be computed later, using both the time-domain sequence technique and the frequency-domain coherence method [50-52]. The HRV time- and frequency- domain parameters for steady state data such as supine rest periods will be computed later according to published standards [53-55]. In addition, time-frequency analysis (change of spectral parameters over time) will be computed for the ACP test [56]. A physician will review the ECG for any abnormal findings and make appropriate clinical recommendations to the subject's referring site physician by phone or make arrangements directly if the subject is a subject of one of the Principal Investigators.

Active Change of Posture (ACP): Subjects will have a 3 to 5-lead Faros ECG monitor placed on them in a private room with white noise and dim lighting (matching lux and dB levels across sites). Subjects will rest supine for 10 minutes followed by another 5 minutes of supine baseline. Subjects will then stand close to their bed within 3 seconds (or as quickly as they can) without reclining on any furniture and 5 more minutes will be recorded. The research coordinator's presence in the room throughout the entire process will ensure compliance with the protocol and also document if the subject reports any symptoms (visits 1-5). We will use the supine baseline for HRV and spontaneous BRS analysis. The ACP procedure results in venous pooling. Characteristic HR change dynamics after stand up can index cardiovagal function, e.g. (R-R interval at beat 30)/(R-R interval at beat 15) [57-59]. The immediate response is a sharp decrease in BP and total systemic resistance at 5 to 10 seconds, followed by a rapid rebound and overshoot. A corresponding HR increase follows in 3 to 5 seconds and then attenuates. After 30 seconds, the hemodynamic parameters become relatively stable [60]. HRV and BP variability provide deeper insight in the time course of the autonomic response to ACP. Total and lf power are raised after standing and hf fraction is reduced [61]. Although complex physiological adjustments occur, the test is easy to administer and repeat by the subjects at home (see below). Time-frequency spectral analysis of HRV and BP data will detect changes in sympathetic and vagal function and BRS. Coded 24-hour HRV and ACP recordings will be sent to Dr. Julian Thayer's research group at University of California Irvine for analysis.

Valsalva Maneuver: Only subjects enrolled at MCW will quantify the cardiovagal/adrenergic components of ascending and descending baroreflex functions. It provides a measure of BRS beyond the spontaneous BRS analysis from ACP [62]. After a 1-minute baseline, the Valsalva is performed by having the subject hold intrathoracic pressure of 40 mmHg for 15 seconds and then release. A series of 4

reproducible maneuvers will be performed, 2 while lying flat and 2 while tilted at 30°. The maneuver is repeated 4 times in total, not including practice runs. The Valsalva Ratio and beat-to-beat BP response are computed. Cardiac sympathetic function is inferred from the resulting increase in HR, while maintenance of mean pressure during the maneuver reflects vasomotor sympathetic function, with gender and age-based norms described for both. The bradycardia that occurs during the release phase represents a second, confirmatory measure of cardiac parasympathetic function [58, 63, 64].

Uroflow and 24h voiding diaries will be performed at major visits (visits 1-5) to evaluate the impact of changes in HRV on bladder physiology. Subjects will be asked to urinate on a special toilet in the FH Neurology Research Rooms during which the speed and pattern of their urine stream will be determined. Subjects will also be asked to monitor their drink intake and output for the same 24 hours they complete the 24-hour EMA. They will measure their own output using a hat provided to them by the research team.

Home checks (figure 1 small up-arrows) will occur once each week, even on-site visit weeks. 24-hour HR recording will use the Faros 360 (Mega Electronics Ltd, Finland) for RR-intervals & accelerometry during the following assigned activities and the surrounding 24-hour periods. On each night, subjects will go to a bedroom with minimal distractions (i.e. no TV or radio, only communication devices to communicate with research coordinator) and with dim lighting to rest supine for 10 minutes followed by another 5 minutes of supine baseline. Subjects will then stand up within about 3 seconds without reclining and stand still for 5 more minutes. The research coordinator will coach via phone or Skype to ensure compliance for the first few recordings, until the subject is comfortable with this process. An accelerometer built into the eMotion HRV 3D device will document position changes. Subjects will complete a weekly Ecological Momentary Assessment (Appendix 2) during the day of monitoring to assess adherence to the at-home ACP tests as well as to record any shifts in mood/affect or subjective pain experience. The 24-hr HR recording will later be analyzed for ANS-R parameters.

Subjects will also complete the GUPI, Promis 8a and 8b, and a limited version of the MPI either online or via paper format. The subject's completed questionnaires and the HR recording will be uploaded to a secure MCW server.

After each site visit and the 24 hours before visit 5, subjects will complete the bedtime ACP recording followed by the 24-hr ANS-R screen, as described earlier, without any questionnaire (except diary as before) to ensure comparability of the HRV recording at home to the one performed at the matching on-site visit. The recorded HR data will be downloaded from the monitor and standard HRV analysis for steady state (supine rest phase of ACP test) and time-varying HRV analysis will be performed, as described above, extending over the 24-hr period, as we have shown [40].

Beta-Blocker vs Placebo

All MPP and IC/BPS subjects in the Experimental Group will participate in this aim occurring simultaneously with aim 1 and employing a 4-8-4-8 week crossover design. A baseline of 4 weekly repeated measures will precede 8 weeks of placebo or beta-blocker, 4 weeks of washout, and 8 additional weeks of crossover. Subjects will be adaptively randomized across all 3 sites in blocks of 4 to either beta-blocker or placebo for ANS-R modulation, stratified by the presence or absence of IC/BPS, using Piantadosi's Randomization software [41], overseen by Dr. Pippa Simpson. We chose a β_1-adrenoceptors cardio-selective blocker because of its more restricted effects. Metoprolol is useful in the

treatment of arrhythmias, hypertension, angina, acute myocardial infarction and the prevention of migraine headaches. Based on personal communication with cardiology and psychiatry (R Josephson 10-15-2014 and J Thayer 9-11-14), 8 weeks are adequate to observe a full effect, and 4 weeks for washout. This intervention aims at our prior cycle finding that subjects with IC/BPS have higher baseline HR compared to HCs. After 4 weeks baseline, subjects will receive a bottle with capsules containing 25 mg of metoprolol tartrate or placebo distributed in a double-blind manner by each site's investigational pharmacy. Subjects will start at 25 mg once daily and increase to the goal dose of 25 mg 2/day after one week, if HR has not decreased below 55 bpm at rest. Subjects will report daily resting HR for the first week. A member of the research team will train the participant how to count her pulse. Chen W et al. [65] reported increased hfHRV and decreased lfHRV in 60 sleep-deprived young adults with this dose, noting a reduction in premature atrial and ventricular complexes. The subjects will then washout for 4 weeks and enter crossover in similar manner (red and green bands in figure 1).

If for some reason it is not possible for the patient to collect the study product while on-site during a study visit, the study drug may be mailed to the patient.

Adverse Events (AEs), Serious Adverse Events (SAEs) and Unanticipated Problems Involving Risks to Human Subjects or Others (UPIRHSOs)

Adverse Events
Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of any study procedure or treatment, regardless of whether it is considered related to the study procedure or treatment.

Serious Adverse Event
Any adverse drug experience that:
- Results in death; OR
- Is life-threatening or places the subject at immediate risk of death; OR
- Results in inpatient hospitalization or prolongation of existing hospitalization; OR
- Results in a persistent or significant disability/incapacity; OR
- Results in a congenital anomaly/birth defect; OR
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Unanticipated Problems Involving Risks to. Human Subjects or Others (UPIRHSOs)
Any incident, experience, or outcome that meets all of the following criteria:
- **Unexpected** in terms of nature, severity, or frequency. (Not found in the current research related documents or in the characteristics of the subject population being studied); AND
- **Related** or possibly related to participation in the research; AND
- Suggests that the research places subjects or others at a greater **risk** of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

Aim 3 Sub-study fMRI

GE 3T MR750 MRI System ***The 3T will be used for this proposal***
Third Generation Magnet Design. Contains 18 superconducting higher-order shim coils, active-shielding

technology, and stainless steel foundation. The magnet's high homogeneity delivers excellent fat-saturation away from isocenter and ensures image quality over a full 48-cm field of view. Zero boil-off technology and remote magnet monitoring technology are incorporated.

High-Performance Whole-Body Gradients. The MR750 incorporates the eXtreme Resonance Module (XRM). XRM gradients deliver 50 mT/m peak amplitude and 200 T/m/s maximum slew rate on each axis simultaneously. The gradients are water-cooled and equipped with integrated thermo-electric cooling panels to provide 100% duty-cycle and stability for advanced studies, such as fMRI. Acoustic noise is minimized, using a passive acoustic barrier material that reduces acoustic transmission by an average of 6 dB for enhanced patient comfort without compromising imaging performance.

Higher-Order Shimming. The MR750 is equipped with five higher-order shim-resistive shim coils to minimize patient-induced field perturbations. In addition to the linear terms (X, Y, Z), the shim set includes XY, XZ, YZ, Y2, X2-Y2 compensation coils.

RF Amplifier. To drive the MR 750 body coil, the system utilizes a water-cooled, solid-state 35 kW RF amplifier design. The amplifier supports a 100 W continuous-wave output mode for advanced proton-decoupling and spin-labeling research.

Host Computing Platform, Reconstruction Engine, and Software. Version 20.x based software. The MR750 utilizes 2.6 GHz processing technology with VRE2.0 recon architecture. With its expansive 32 GB memory, acquisition-to-disk technology, and 5400 2D FFT/s frame rate, the VRE 2.0 delivers the processing power to reconstruct 32 channels of high-resolution 3D volumetric data.

This 3T scanner enhances the shared use of the centralized MRI resources, enrich ongoing research, promote new research directions, foster a cooperative and interactive research environment, and stimulate multidisciplinary approaches to neuroscience. MCW has provided strong institutional commitment toward the 3T imaging facility and maintenance.

Version-20.x-based software will include all commercially available pulse sequences, spectroscopy, and imaging options, nominally equivalent in form and function to the MR750 (3T) platform. Dual CPU host-computer includes Linux OS, dualcore.

Diffusion Kurtosis Imaging (DKI) scans use the SE-EPI pulse sequence with multiple non-collinear gradient directions and at least two b values such as 1000 and 2000, 4 reference scans will also be acquired in the axial orientation, aligned with the high resolution T1 weighted scans (8 minutes). Motion and eddy current related distortions will be corrected with FSL software (http://www.fmrib.ox.ac.uk/fsl/) [66, 67]. DKI data will be processed using software developed in-house that generates axial, radial and mean kurtosis maps (Kax, Krad and MK) in addition to conventional Fractional Anisotropy and Mean Diffusivity (FA and MD) maps. DKI measures non-Gaussianity of the diffusion signals and it has been shown to be a more sensitive marker of subtle changes in brain white matter compared to conventional DTI [68].

Spectroscopy of PAG: single voxel magnetic resonance spectroscopy of the PAG to investigate relative distribution of brain metabolite spectra in the PAG of patients with CPP.

Resting-State fcMRI Acquisition. Subjects stay awake, eyes open, wakefulness monitored on a 10-point Likert scale, 1 ("very sleepy") to 10 ("very alert"). T2*-weighted functional images will be acquired with a gradient-echo EPI sequence using 45 interleaved axial contiguous 2.5-mm thick slices, along with respiratory and cardiac signals (2 imaging runs of 132 volumes and 6.5' each). Dr Lisa Conant will do pre-scan coaching in the mock MCW scanner and during scanning.

G. RISKS AND THE PRECAUTIONS WHICH WILL BE TAKEN TO MINIMIZE RISK EXPOSURE

Overall, potential risks associated with participation in the proposed study are similar to those experienced by women undergoing standard of care for chronic pelvic pain.

Physical. Subjects participating in the phenotyping portion of the proposed research (Aim 1) will be exposed to physical risks associated with the standard of care for chronic pelvic pain, including but not limited to pelvic examination. The course of treatment is left to the discretion of each subject's urologist, urogynocologist or pelvic pain specialist. In addition to the standard of care, research subjects will undergo non-invasive testing such as tender point evaluation, baroreflex and HRV assessment as part of study screening, baseline metrics, and follow-up visits. Tender point evaluation is considered standard of care for myofascial pain disorders such as fibromyalgia and may lead to bruising and pain at the site of palpation. Procedures used to assess baroreflex sensitivity and HRV are considered the standard of care for assessing autonomic nervous system deficiencies with minimal associated physical risks. Subjects may feel anxious, dizzy, nauseated or get a headache from breathing in and out as instructed during the breathing test. (HR and blood pressure monitoring during structured breathing and change of posture). Use of beta-blockers (Aim II) such as metoprolol can lead to diarrhea, stomach cramping, nausea, vomiting, rashes, blurred vision, muscle cramping, fatigue, depression, heart failure or heart block in subjects with heart problems (although known heart issues are an exclusion criteria), headaches, dizziness, nightmares, hallucinations, shortness of breath in asthmatics, sexual dysfunction, and may cause low or high blood glucose. Physical risks associated with fMRI (Aim III) are not fully known but current evidence indicates that there is minimal risk associated with the procedure. Because the MRI scanner produces a very strong magnetic field, there is some risk involved in the form of the potential for metallic objects to move through the room creating projectiles that could harm individuals and/or damage the scanner. Healthy controls will be screened for the absence of pelvic pain, and other pain/autonomic disorders, baseline metrics, psychological questionnaires will be determined, and subjects may partake in the HRV, baroreflex, and fMRI portions of the study. Potential physical risks will be discussed with all subjects and knowledge of these risks will be made evident through the consent form.

Psychological. Risks associated with the psychological questionnaires are considered minimal. Since self-disclosure of personal information may produce some transient emotional discomfort, the informed consent process makes clear that subjects may decline to participate in any aspect of the project, including completing questionnaires. Although not a direct risk of the study, our instruments may expose levels of psychopathology that require clinical attention. Measures will be scored within 1 week and subjects whose scores exceed established cutoffs will be discussed and the senior investigator will be notified. Contact of the subject will be at the discretion of the principal investigator. If necessary, the subject will be provided a referral for further psychological or psychiatric evaluation.

H. PROVISION FOR THE PROTECTION OF PRIVACY OF SUBJECTS AND TO MAINTAIN THE CONFIDENTIALITY OF DATA

All research projects that collect electronic data must use appropriate security measures to ensure that data is protected from theft or loss in order to prevent breaches of confidentiality. You must indicate what encryption tools (or why they are not necessary) from the options below. The IRB will not review this protocol unless you indicate the encryption tools being used to secure your research data. If you do not have encryption in place on your systems, please contact your Information Systems support to arrange for one of the encryptions options listed below.

The following encryption products employ cryptographic modules that the National Institute of Standards and Technology has certified as meeting FIPS 140-2 requirements. Children's Hospital and Health System endorsed the use of these products made to encrypt hard drives and removable media. All electronic research data must be encrypted using one or more of these products.

Please indicate which encryption tools you are using to secure your research data.

___ Credent Mobile Guardian (RS, PD)
___ GuardianEdge Hard Disk and GuardianEdge Removable Storage Encryption (HD, RS, PD)
_X_ IronKey encrypted flash drives (RS)
___ McAfee Endpoint Encryption (HD, RS)
___ Microsoft Bitlocker (HD, RS when used with Windows 7 and FIPS compliant algorithms are enabled)
___ PGP Whole Disk Encryption and PGP Portable (HD, RS)
___ SafeNet Protect Disk and SafeNet Protect File (HD, RS)
___ Seagate Secure Self-Encrypting Drives (HD when encryption option is enabled)
___ Symantec Endpoint Encryption (HD, RS, PD)
_X_ WinMagic SecureDoc encryption (HD) (for MCW owned computers)
_X_ Other: Trucrypt

Does not apply because:

___ Data is de-identified – no PHI collected
___ Data is stored on paper only
___ Data is stored on CHW secured shared drives.
_X_ Data is stored on MCW secured shared drives.

Key

HD = Hard Drive
RS = Removable Storage (USB flash drive, CD, etc.)
PD = Portable Device (iPod; iPhone; PDA, etc.)

I. PROVISIONS FOR MONITORING DATA TO ENSURE THE SAFETY OF SUBJECTS; AND ADDITIONAL SAFEGUARDS TO PROTECT THE RIGHTS AND WELFARE OF SUBJECTS WHO ARE LIKELY TO BE VULNERABLE

The Principal Investigator will monitor the health of all subjects in this study per standard clinical practice. Subjects who are found to have suicidal ideation will be contacted by study co-investigator and psychologist, Dr. Lisa Conant. Physician referrals will be provided as needed and as dictated by law to

ensure subject and family safety and to address psychosocial needs. The Principal Investigator will monitor protocol adherence and supervise data collection, entry, and analysis. The Research Coordinators will share their contact information with each subject in case of study-related emergency. If the Coordinator is contacted, they will direct the subject accordingly and contact the Principal Investigator immediately.

J. ANTICIPATED BENEFITS ASSOCIATED WITH THE PROTOCOL TO HUMAN RESEARCH SUBJECTS AND SOCIETY

The anticipated risks to subjects are reasonable in relation to the anticipated benefits and the importance of the knowledge that is expected to result from the study. While there may be no direct benefit of the study to subjects, the study has the potential of benefit of increasing our knowledge of factors contributing to chronic pelvic pain. Although this study does not focus on any specific treatment and does not depend on treatment fidelity, it will nonetheless benefit from this standard approach.

K. STOPPING POINTS THAT WOULD NOT ALLOW THE STUDY TO CONTINUE AS PROPOSED

The *Primary Endpoint* is whether change in ANS-R is accompanied by a change in the connectivity between PFC and PAG. We will use a linear model with connectivity at 24 weeks as outcome, connectivity at baseline as a covariate and change in ANS-R, demographics such as age and group and the interactions of group with other covariate.

The *Secondary Endpoints* are to ascertain structural changes that correlate with larger ANS-R, 1) changes in fiber bundles connecting PFC and PAG using DTI/DKI, and 2) changes in the size of PAG using VBM analysis. We expect to see changes in structure at 24 weeks parallel with ANS-R change.

L. IS THERE A DATA SAFETY MONITORING BOARD IN PLACE? WHO ARE ITS MEMBERS? HOW OFTEN DO THEY MEET?

A DSMB of independent investigators will be established to advise the NIDDK and the study investigators. Their responsibilities include the annual review and evaluation of the accumulated study data for subject safety, study conduct and progress, and, to make recommendations to NIDDK concerning the continuation, modification, or termination of the trial. They will monitor adverse events, evaluate data quality, completeness and timeliness, examine performance of the individual sites, review adherence to the protocol and intervention fidelity across site, and oversee factors that might affect subject safety or data confidentiality. We will recruit national experts in CPP or autonomic function to be on the ICECAN advisory board and DSMB. All key investigators and the DSMB will meet once per year. Any severe adverse events (SAEs) encountered that the principal investigator believes are severe and related to the study medication will be presented to the Board and reported to the IRB at the time of the occurrence. All other SAEs and adverse events (AEs) will be recorded and submitted annually to the DSMB.

M. DESCRIBE HOW THE CONSENT PROCESS WILL TAKE PLACE. INCLUDE A LIST OF APPROPRIATELY TRAINED PERSONNEL WHO WILL BE INVOLVED

Written informed consent for participation will be obtained from the subjects. Consent will be obtained by a study investigator or a CITI trained research team member at the subject's OB/GYN or Neurology Clinic appointment in FMLH, either at the main campus or at a satellite clinic. The subject's consent will allow for accessing information collected for program evaluation/clinical purposes. Participating

volunteers will have the option of having the consent document read aloud to them to facilitate understanding. Copies of signed consent document will be given to subjects. Consent will be obtained by the study principal investigator, co-investigators or other CITI trained research staff. As additional research staff or team members are added, their names will be submitted to the IRB as a protocol amendment to allow them to obtain consent.

Subjects who agree to the optional blood draws will initial the corresponding field on the consent form. Subjects who agree to provide the optional blood samples will be asked at each visit if they still agree to the blood collection.

N. PROCEDURES TO BE EMPLOYED IN ANALYZING DATA AND THE ANTICIPATED SIGNIFICANCE OF THE PROPOSED STUDY

Power: There is little prior information on which to perform a formal power analysis, so conservative assumptions determine the difference in correlations that will be detectable with 80% power at the .017% level (to allow for 3 independent tests – and more dependent ones – at the 5% level). We assume an analysis of 102 subjects allowing for 15% dropout in which partial correlations (partialing out IC/BPS versus MPP and other relevant covariates i.e., age and treatment are analyzed. First, we use conservative assumptions to determine the difference in correlations that will be detectable with 80% power at the .017% level (to allow for 3 independent tests at the 5% level). Assuming an effective sample size of 102-3 =99 , the z-transform of a correlation will then have variance 1/96 (90) and the difference between two such correlations will be twice that, conservatively assuming zero correlation between the two correlations It then follows that, for a two-sided test, a difference in correlation of ~0.4 will be detectable. However, because we expect a strong positive correlation between the two correlations, a much smaller difference will be detectable.

*Analysis***:** Standard mixed model regression analysis will determine how well baseline measures predict later measures. Since all the measures will be continuous or quasi-continuous, all data will be transformed, as necessary, for residuals to be approximately normally distributed. Although both predictors of interest and possible confounding covariates can be simultaneously included in the regression models, examining the outcome variables univariately or multivariately, to examine the order of time changes, we propose correlation of covariate-adjusted residuals using Fisher's variance-stabilizing z-transformation, appropriately allowing for the correlation between correlations [69]. First, we will take a weighted average of the covariate-adjusted ANS-R variables to minimize the standard error of the weighted average (subject to the sum of the squared weights equaling 1), and similarly for the pain scores. With 5 major time points there are 4-time intervals. We define a change as the (signed) difference in value of a variable between two time points, so that for each variable we have 10 changes (4 of length 1, 3 of length 2, 2 of length 3, and 1 of length 4 intervals). We thus have a 10x10 matrix of correlations when we pair a change in the ANS-R variable with a change in the clinical score: 11 of these correlations correspond to changes in ANS-R preceding changes in pain, and 11 to changes in pain preceding ANS-R: the other 78 correlations are ambiguous regarding the order in which the changes occur (Table 3). We will calculate these 22 correlations and, separately in each of the three groups, test the equality of the 11

Interval comparisons	Changes in HRV									
	1→2	2→3	3→4	4→5	1→3	2→4	3→5	1→4	2→5	1→5
1→2		1•1	1•1	1•1		1•2	1•2		1•3	
2→3	1•1		1•1	1•1			1•2			
3→4	1•1	1•1		1•1						
4→5	1•1	1•1	1•1							
1→3							2•2			
2→4	2•1									
3→5	2•1	2•1			2•2					
1→4										
2→5	3•1									
1→5										

Changes in Pain

Table 3. In each of the 22 cells for which there is no ambiguity regarding the sequence in which changes occur, the entry *a•b* is a pair of (major) interval lengths*: a* for changes in pain occurring first, *b* for changes in HRV occurring first.

implying pain causes ANS-R to the 11 implying the converse, performing three two-sided tests: one on the average correlations where all intervals are of length 1; one on the average of those where the interval length is 1 for one variable and 2 for the other; and one for the correlations where the interval length is 2. If one of these is significant, similar detailed analyses with finer intervals will be warranted.

The *Primary Endpoint* of aim 1 is the determination of time sequence between changes in ANS-R and changes in measures of daily pain and function (MPI, Promis 8a/8b, GUPI). Secondarily, we will also assess sequence relationships between changes in ANS-R and changes in bladder function by the same methodology. *We expect ANS-R, a putative measure of the operating mode of vmPFC, to increase (both in total power and in HF fraction) before clinical improvement in subjects who fulfill the IC/BPS phenotype*. **Pitfalls and alternate approaches**: if we do not demonstrate this specific relationship: 1) a different relationship may emerge, and we will explore its implications; 2) the relationship may exist in certain subgroups, for example predicted by other aspects of baseline phenotype such as ANS-R, or psychological features; 3) the relationship may exist only for major changes in clinical status as may happen during "flares" or "remissions"; 4) specific aspects of ANS-R responsiveness (such as change with standing) may correlate better with clinical change than ANS-R itself in a particular state.

Secondary Endpoint: 1) determination of whether ANS-R at baseline predicts clinical status (MPI, GUPI), bladder physiology, at 4, 12, 16 and 24 weeks. **Pitfalls and alternate approaches**: if this is not the case, we will examine other psychological measures for a similar predictive role, and ANS-R stimulus responsiveness rather than ANS-R itself.

Technical Considerations: though this portion is not invasive and virtually without associated risks for the subjects, we may still struggle with poor home screen compliance. If so, we have considered sending the research coordinators to the homes (if budget allows) or change from weekly visits to every other week.

Aim 3 Analysis, Endpoints, Expected Findings, Pitfalls and Alternate Approaches

Analysis: **VBM:** Voxel based lesion-symptom mapping studies suggest a major role in organizing and planning goal-directed behavior for lPFC regions, while vmPFC provides insight and harmonizes behavior with emotional, social and cognitive forces (see for example [70]). One might speculate that goal-directed behavior from lPFC regions must suppress ascending signals through PAG, while internally attentive behavior (through vmPFC) will want to enhance these signals to attend to them, potentially explaining the opposing forces these two areas exert over PAG. We will be examining these relationships in great detail across our subjects. VBM will be compared between HCs, IC/BPS and MPP subjects, initially; paired sampling across time will be utilized to determine the change in PAG size across time; the magnitude of this change will be regressed against quantitation of improvement using mixed models as described in aim 2. **DTI/DKI:** Kax, Krad, MK, FA and MD maps will be generated for statistical analysis, and changes in both associated with HRV index changes in tracts that connect the PFC to PAG and raphe will be analyzed using Tract-Based Spatial Statistics (TBSS), a non-parametric permutation test for analysis [71, 72]. Statistical tests will be thresholded at $p<0.01$ corrected for multiple comparisons using family-wise error rate. *We will test the hypothesis that the connecting bundles enlarge in those subjects whose HRV increases across the 3 time points*. **Connectivity:** After standard image pre-processing using the AFNI software package [73], cardiac and respiratory cycle noise removal [74], band-pass filtering, Voxelwise multiple linear regression analysis (AFNI's 3dDeconvolve [75]), we will analyze the correlation BOLD time-course coefficient maps for each

region of interest (ROI), and convert to Z-score maps. An automated affine transformation maps Z-score maps to stereotaxic atlas space, with standard smoothing and calculation of t-statistics for each ROI. Significance uses magnitude and extent (cluster size) criteria with permutation testing to select a whole-brain corrected $p < 0.01$ threshold, along with ordinary least square to account for covariates. Changes in functional connectivity strength between PAG and PFC associated with HRV changes will be analyzed using resting state fcMRI results. Temporal data analysis will utilize 3dREMLfit (http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dREMLfit.html). CSF pulsation and respiration signal contamination will be minimized by regressing out signals that correlate with EKG and respiratory cycles prior to rs-fMRI analysis. These are recorded with MRI compatible EKG system and a respiratory belt.

Sample size justification: For a correlation of 0.5 with 18 in the sample, we will have 95% confidence interval of (0.043, .784). We will examine the change over time using a mixed effects model. We expect that brain maps of connectivity will show such a change, present when HRV has significantly increased, and more marked in IC/BPS than in MPP. **Pitfalls and alternate approaches**: connectivity changes may occur in other areas seeding from PAG. We will use the information to generate hypotheses regarding the underlying neurophysiology of IC/BPS. If the changes do not correlate with ANS-R change, we will re-explore the analysis using clinical improvement.
Pitfalls and alternate approaches: should we not see changes in subjects with better ANS-R, we may consider extending the fMRI and DKI portion of the grant.

Timeline for all Aims	Year 1				Year 2				Year 3				Year 4				Year 5			
Specific Aims	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
AIM 1: Observational Study																				
Clinical setup																				
Enroll patients																				
Data analysis & Manuscript preparation																				
AIM 2: HRV Modulation																				
Clinical & Pharmacy setup																				
Enroll patients																				
Data analysis & Manuscript preparation																				
AIM 3: fMRI and DTI																				
Protocol setup																				
Enroll patients																				
Data analysis & Manuscript preparation																				

Table 4. Projected study timeline for Aims 1-3.

Timeline (Table 4) and Data Management. The NIH-MAPP data core at U Penn will manage all subject data while the UCLA imaging core will manage imaging data. We will use REDCap onsite (Research operates within the Secure Socket Layer (SSL) 128-bit encryption Electronic Data Capture) a secure, web-based application designed for research data capture. Clinical data entry will occur on site and MCW will be responsible for the physiologic data entry. Sites will only see data from their site and access privileges will reflect need for and is fully in compliance with: (a) Guidance for Industry 21 CRF Part 11; Electronic Records; Electronic Signatures; Scope and Application [FDA]; (b) 21 CRF Part 820 (QSR); (c) Guidance for Industry; E6 Good Clinical Practice: Consolidated Guidance; (d) Guidance for Industry; Computerized Systems used in Clinical Investigations; (e) General Principals of Software Validation; Final Guidance for Industry and FDA Staff; (f) Health Insurance Portability and Accountability Act of 1996 (HIPAA).
Missing Data. We will examine the pattern of missing data using logistic regression. Where it seems reasonable to assume the data are missing at random (MAR), we will use a mixed model approach or for any items missing such as demographics, we will use multiple imputation. We will conduct a sensitivity analysis to check other assumptions, if it appears data are not MAR.

O. FINANCIAL RELATIONSHIPS

This protocol is supported by a NIH grant. This grant provides funding for study related procedures (HRV, questionnaires, physician examinations, Valsalva maneuver), data analysis and study incentives to subjects.

P. ADVERTISEMENTS / FLIERS

Flyers, advertisements and MCW fMRI Bank (for MCW only) will be used to recruit healthy control subjects. Flyers will be posted throughout the Clinic Workrooms in FMLH and specialty clinics. Recruitment flyers will also be posted at Marquette University, University Wisconsin Milwaukee, and local churches. Advertisements will include an information page placed on the Medical College of Wisconsin's Infoscope informational page as well as The Interstitial Cystitis Association web site (https://www.ichelp.org/research/clinical-trials/studies-seeking-patients/).

Experimental subject recruitment flyers, and supplemental online pre-screen/ QR code ad will also be posted at area Universities within a 150-mile radius of MCW (ex. Marquette, UW-Milwaukee, UW-Madison), area bus lines (ex. Metro, Badger bus), Wisconsin Athletic Clubs in the area, local churches, and local YMCAs. These flyers will only be posted after the institution/company gives us the approval to do so. This flyer will also be posted on Facebook and subjects will be encouraged to call or email the study team.

Experimental subject supplemental online pre-screen/ QR code will also be posted at area universities, bus lines, athletic clubs within a 150-mile radio of Northshore University in Illinois.

Additional advertisement will be used in the form of a TV ad, radio, and possible guest on Milwaukee Public Radio.

Q. BIBLIOGRAPHY

1. Fall, M., et al., *EAU guidelines on chronic pelvic pain.* Eur Urol, 2010. **57**(1): p. 35-48.
2. Mathias, S.D., et al., *Chronic pelvic pain: prevalence, health-related quality of life, and economic correlates.* Obstet Gynecol, 1996. **87**(3): p. 321-7.
3. Stein, S.L., *Chronic pelvic pain.* Gastroenterol Clin North Am, 2013. **42**(4): p. 785-800.
4. Berry, S.H., et al., *Prevalence of symptoms of bladder pain syndrome/interstitial cystitis among adult females in the United States.* J Urol, 2011. **186**(2): p. 540-4.
5. El Khoudary, S.R., et al., *Severity of Interstitial Cystitis Symptoms and Quality of Life in Female Patients.* Journal of Womens Health, 2009. **18**(9): p. 1361-1368.
6. Beckett, M.K., et al., *Consequences of interstitial cystitis/bladder pain symptoms on women's work participation and income: results from a national household sample.* J Urol, 2014. **191**(1): p. 83-8.
7. Suskind, A.M., et al., *Health-related quality of life in patients with interstitial cystitis/bladder pain syndrome and frequently associated comorbidities.* Qual Life Res, 2013. **22**(7): p. 1537-41.
8. Anger, J.T., et al., *Treatment choice, duration, and cost in patients with interstitial cystitis and painful bladder syndrome.* Int Urogynecol J, 2011. **22**(4): p. 395-400.
9. As-Sanie, S., et al., *Changes in regional gray matter volume in women with chronic pelvic pain: A voxel-based morphometry study.* Pain, 2012. **153**(5): p. 1006-1014.
10. Chalaye, P., et al., *Comparing pain modulation and autonomic responses in fibromyalgia and irritable bowel syndrome patients.* Clin J Pain. **28**(6): p. 519-26.

11. Meeus, M., et al., *Heart rate variability in patients with fibromyalgia and patients with chronic fatigue syndrome: a systematic review.* Semin Arthritis Rheum. **43**(2): p. 279-87.
12. Shiro, Y., et al., *Effect of muscle load tasks with maximal isometric contractions on oxygenation of the trapezius muscle and sympathetic nervous activity in females with chronic neck and shoulder pain.* BMC Musculoskelet Disord. **13**: p. 146.
13. Gockel, M., et al., *Perceived disability but not pain is connected with autonomic nervous function among patients with chronic low back pain.* J Rehabil Med, 2008. **40**(5): p. 355-8.
14. Mazurak, N., et al., *Heart rate variability in the irritable bowel syndrome: a review of the literature.* Neurogastroenterol Motil. **24**(3): p. 206-16.
15. Ness, T.J., L.K. Lloyd, and R.B. Fillingim, *An endogenous pain control system is altered in subjects with interstitial cystitis.* J Urol. **191**(2): p. 364-70.
16. Cook, J., Bigger, JT, Kleiger, RE, Fleiss, JL, Steinman, RC, Rolnitzky, LM, *Effect of atenolol and diltiazem on heart period variability in normal persons.* J Am Coll Cardiol, 1991. **17**(2): p. 480-484.
17. Felber Dietrich, D., et al., *Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study.* Europace, 2006. **8**(7): p. 521-9.
18. Aronson, D. and A.J. Burger, *Effect of beta-blockade on heart rate variability in decompensated heart failure.* Int J Cardiol, 2001. **79**(1): p. 31-9.
19. Sandrone, G., et al., *Effects of beta blockers (atenolol or metoprolol) on heart rate variability after acute myocardial infarction.* Am J Cardiol, 1994. **74**(4): p. 340-5.
20. Lampert, R., et al., *Effects of propranolol on recovery of heart rate variability following acute myocardial infarction and relation to outcome in the Beta-Blocker Heart Attack Trial.* Am J Cardiol, 2003. **91**(2): p. 137-42.
21. Niemele, M., Airaksinen, KE, Huikuri, HV, *Effect of beta-blockade on heart rate variability in patients with coronary artery disease.* J Am Coll Cardiol, 1994. **23**(6): p. 1370-1377.
22. Clauw, D.J., et al., *The relationship between fibromyalgia and interstitial cystitis.* J Psychiatr Res, 1997. **31**(1): p. 125-31.
23. Ness, T.J., et al., *Psychophysical evidence of hypersensitivity in subjects with interstitial cystitis.* J Urol, 2005. **173**(6): p. 1983-7.
24. Tracey, I. and P.W. Mantyh, *The cerebral signature for pain perception and its modulation.* Neuron, 2007. **55**(3): p. 377-91.
25. Hadjipavlou, G., et al., *Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: a diffusion tensor imaging study in healthy controls.* Pain, 2006. **123**(1-2): p. 169-78.
26. Schweinhardt, P., et al., *Investigation into the neural correlates of emotional augmentation of clinical pain.* Neuroimage, 2008. **40**(2): p. 759-66.
27. Mayer, E.A., et al., *Differences in brain responses to visceral pain between patients with irritable bowel syndrome and ulcerative colitis.* PAIN, 2005. **115**(3): p. 398-409.
28. Brighina, F., et al., *rTMS of the prefrontal cortex in the treatment of chronic migraine: a pilot study.* J Neurol Sci, 2004. **227**(1): p. 67-71.
29. Apkarian, A.V., et al., *Chronic back pain is associated with decreased prefrontal and thalamic gray matter density.* J Neurosci, 2004. **24**(46): p. 10410-5.
30. Lane, R.D., et al., *Neural correlates of heart rate variability during emotion.* Neuroimage, 2009. **44**(1): p. 213-22.

31. Roy, M., D. Shohamy, and T.D. Wager, *Ventromedial prefrontal-subcortical systems and the generation of affective meaning.* Trends Cogn Sci, 2012. **16**(3): p. 147-56.
32. Benarroch, E.E., *Periaqueductal gray: an interface for behavioral control.* Neurology, 2012. **78**(3): p. 210-7.
33. Keay, K.A. and R. Bandler, *Parallel circuits mediating distinct emotional coping reactions to different types of stress.* Neurosci Biobehav Rev, 2001. **25**(7-8): p. 669-78.
34. Pereira, E.A., et al., *Ventral periaqueductal grey stimulation alters heart rate variability in humans with chronic pain.* Exp Neurol, 2010. **223**(2): p. 574-81.
35. Holstege, G., *The emotional motor system and micturition control.* Neurourol Urodyn. **29**(1): p. 42-8.
36. Griffiths, D., et al., *Brain control of normal and overactive bladder.* J Urol, 2005. **174**(5): p. 1862-7.
37. Griffiths, D., et al., *Cerebral control of the bladder in normal and urge-incontinent women.* Neuroimage, 2007. **37**(1): p. 1-7.
38. Kuhtz-Buschbeck, J.P., et al., *Cortical representation of the urge to void: a functional magnetic resonance imaging study.* J Urol, 2005. **174**(4 Pt 1): p. 1477-81.
39. Disorders, T.I.C.o.E.-D.S.R., *EDS Diagnostics*, D.C.f.H.E.-D.S. (hEDS), Editor. 2017, The Ehlers-Danlos Society.
40. Welzig, C.M., et al., *Lipid lowering by pravastatin increases parasympathetic modulation of heart rate: Galpha(i2), a possible molecular marker for parasympathetic responsiveness.* Circulation, 2003. **108**(22): p. 2743-6.
41. Piantadosi, S., *Randomization ©. Block randomization software windows version 6.0*. 2011, Cedars-Sinai Medical Center: Los Angeles, CA.
42. Spielberger CD SS, W.A., MArsch BJ, *Measuring anxiety and anger with the State-Trait Anxiety Inventory (STAI) and the State-Trait Anger Expression Inventory (STAXI)*, in *The Use of Psychological Testing for Treatment Planning and OUtcomes Assessment*, M. M, Editor. 1999, awrence Erlbaum Associates, Inc: Hahwah, NJ.
43. Pennebaker, J., *The Psychology of Physicial Symptoms*. 1982, New York: Springer-Vale.
44. Kerns, R.D., D.C. Turk, and T.E. Rudy, *The West Haven-Yale Multidimensional Pain Inventory (WHYMPI).* Pain, 1985. **23**(4): p. 345-56.
45. Clemens, J.Q., et al., *Validation of a Modified National Institutes of Health Chronic Prostatitis Symptom Index to Assess Genitourinary Pain in Both Men and Women.* Urology, 2009. **74**(5): p. 983-987.e3.
46. Clemens, J.Q., et al., *Validation of a Modified National Institutes of Health Chronic Prostatitis Symptom Index to Assess Genitourinary Pain in Both Men and Women.* Urology, 2009. **74**(5): p. 983-987.
47. Cohen, S., T. Kamarck, and R. Mermelstein, *A global measure of perceived stress.* J Health Soc Behav, 1983. **24**(4): p. 385-96.
48. Omboni, S., et al., *Spectral and sequence analysis of finger blood pressure variability. Comparison with analysis of intra-arterial recordings.* Hypertension, 1993. **22**(1): p. 26-33.
49. Parati, G., et al., *Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing.* Hypertension, 1989. **13**(6 Pt 1): p. 647-55.
50. Parati, G., M. Di Rienzo, and G. Mancia, *How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life.* J Hypertens, 2000. **18**(1): p. 7-19.
51. Persson, P.B., et al., *Time versus frequency domain techniques for assessing baroreflex sensitivity.* J Hypertens, 2001. **19**(10): p. 1699-705.

52. Westerhof, B.E., et al., *Time-domain cross-correlation baroreflex sensitivity: performance on the EUROBAVAR data set.* J Hypertens, 2004. **22**(7): p. 1371-80.
53. *Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.* Circulation, 1996. **93**(5): p. 1043-65.
54. Berntson, G.G., et al., *Heart rate variability: origins, methods, and interpretive caveats.* Psychophysiology, 1997. **34**(6): p. 623-48.
55. Acharya UR, J.K., Kannathal N, Lim CM, Suri JS, *Heart rate variability: a review.* Med Biol Eng Comput, 2006. **44**(12): p. 1031-51.
56. Tarvainen, M.P., et al., *Time-varying analysis of heart rate variability signals with a Kalman smoother algorithm.* Physiol Meas, 2006. **27**(3): p. 225-39.
57. Ewing, D.J., et al., *Immediate heart-rate response to standing: simple test for autonomic neuropathy in diabetes.* Br Med J, 1978. **1**(6106): p. 145-7.
58. Low PA, B.E., *Laboratory Evaluation of Autonomic Failure*, in *Clinical Autonomic Disorders*. 2008.
59. Smith, J.J., C.M. Porth, and M. Erickson, *Hemodynamic response to the upright posture.* J Clin Pharmacol, 1994. **34**(5): p. 375-86.
60. Wieling, W., et al., *Initial orthostatic hypotension: review of a forgotten condition.* Clin Sci (Lond), 2007. **112**(3): p. 157-65.
61. Sanderson, J.E., et al., *Impact of changes in respiratory frequency and posture on power spectral analysis of heart rate and systolic blood pressure variability in normal subjects and patients with heart failure.* Clin Sci (Lond), 1996. **91**(1): p. 35-43.
62. Yang, H. and J.R. Carter, *Baroreflex sensitivity analysis: spontaneous methodology vs. Valsalva's maneuver.* Clin Auton Res. **23**(3): p. 133-9.
63. Levin, A.B., *A simple test of cardiac function based upon the heart rate changes induced by the Valsalva maneuver.* Am J Cardiol, 1966. **18**(1): p. 90-9.
64. Smith, M.L., et al., *Valsalva's maneuver revisited: a quantitative method yielding insights into human autonomic control.* Am J Physiol, 1996. **271**(3 Pt 2): p. H1240-9.
65. Chen, W.R., et al., *Protective effect of metoprolol on arrhythmia and heart rate variability in healthy people with 24 hours of sleep deprivation.* J Interv Card Electrophysiol. **36**(3): p. 267-72; discussion 272.
66. Behrens, T.E., et al., *Characterization and propagation of uncertainty in diffusion-weighted MR imaging.* Magn Reson Med, 2003. **50**(5): p. 1077-88.
67. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL.* Neuroimage, 2004. **23 Suppl 1**: p. S208-19.
68. Jensen, J.H., et al., *Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging.* Magn Reson Med, 2005. **53**(6): p. 1432-40.
69. Elston, R., *On the Correlation Between Correlations.* Biometrika, 1975. **62**: p. 133-140.
70. Robinson H, C.M., Glascher J, Bruss J, Tranel D, *Neuroanatomical Correlates of Executive Functions: A Neuropsuchological Approach Using the EXAMINER Battery.* Journal of the International Neuropsychological Society
2013. **19**: p. 1-12.
71. Nichols, T.E. and A.P. Holmes, *Nonparametric permutation tests for functional neuroimaging: a primer with examples.* Hum Brain Mapp, 2002. **15**(1): p. 1-25.
72. Smith, S.M., et al., *Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data.* Neuroimage, 2006. **31**(4): p. 1487-1505.

73. Cox, R.W., *AFNI: software for analysis and visualization of functional magnetic resonance neuroimages.* Comput Biomed Res, 1996. **29**(3): p. 162-73.
74. Glover, G.H., T.Q. Li, and D. Ress, *Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR.* Magn Reson Med, 2000. **44**(1): p. 162-7.
75. Ward, B.D. and Y. Mazaheri, *State-space estimation of the input stimulus function using the Kalman filter: a communication system model for fMRI experiments.* J Neurosci Methods, 2006. **158**(2): p. 271-8.

Appendix 1 Restricted and Prohibited Medications

The following medications are Prohibited while subjects are actively receiving treatment with metoprolol/ placebo:

- Amiodarone (Pacerone), Ceritinib (Zykadia), Clonidine (Catapres), Ergotamine (Cafergot, Ergomar), Other Ergots, Floctafenine (NSAID), Propafenone (Rhthmol), Reserpine, Rivastigmine (Exelon), insulin, verapamil, diltiazem, monoamine oxidase inhibitors
- Other beta-blockers: ((Acebutolol (Sectral), Atenolol (Tenormin), Betaxolol (Kerlone), Bisoprolol (Zebeta), Carteolol (Ocupress), Carvedilol (Coreg), Esmolol (Brevibloc), Labetalol (Trandate), Nadolol (Corgard), Nebivolol (Bystolic), Penbutolol (Levatol), Pindolol (Visken), Propranolol (Inderal), Sotalol (Betapace), Timolol (Blocadren, Betimol), arformoterol, bambuterol, clenbuterol, formoterol, salmeterol)
- Anticholinergic medications

The following medications are Restricted—allowed while subjects are actively receiving treatment with metoprolol/ placebo, but should be used with caution. Monitor subject's HR closely.

- Cholinergics: Benztropine (Cogentin), Methacholine (Provocholine), Orphenadrine (Norflex)
- Antivertigo agents: Meclizine (Dramamine, Bonine), Scopolamine (Transderm Scop.)
- Urinary incontinence agents: Oxybutynin (Ditropan), Solifenacin (VESIcare), Tolterodine (Detrol)
- GI Medications: Dicyclomine (Bentyl), Hyoscyamine (Levsin), Diphenoxylate (Lomotil)
- H1 Blockers: Chlorpheniramine (Chlor-Trimeton), Diphenhydramine (Benadryl)
- Antipsychotics: Chlorpromazine (Thorazine), Clozapine (Clozaril), Olanzapine (Zyprexa), Prochlorperazine (Compro)
- Muscle Relaxants: Dantrolene (Dantrium), Orphenadrine (Norflex)
- Strong 2D6 Inhibitors: Bupropion (Wellbutrin), Cinacalcet (Sensipar), Fluoxetine (Prozac), Paroxetine (Paxil), Quinidine (Quinidex)

Appendix 2 Ecological Momentary Assessment

Sample COMPUTER-GENERATED EMA survey set

PROMPT EACH DAY AROUND 6pm (coordinator can change this time if easy to program)
WILL YOU BE STARTING YOUR WEEKLY RECORDING THIS EVENING?
YES -> initiate program
NO -> repeat prompt next day
NOT SURE -> contact coordinator

QUESTIONS Upon waking:

When did you lay down to go to bed last night? _____ am / pm

How long did it take you to fall asleep last night?
NUMBER OF MINUTES _____

When did you get up this morning? _____ am / pm

----------NEXT SCREEN

SLEEP

Last night, how many times did you awaken? Pull down 0- 5+

If above > 0:

Which factors below was the biggest contributor to your awakenings?

Urination
Pain
Anxiety
Other

Perhaps have the same pull-down 4 times with
Biggest reason
Second biggest reason
Third biggest reason
Fourth biggest reason

Last night, did you take medicine (prescribed or over the counter) to help you sleep? (yes/no)

Which ones?_____(open space to type)
----------NEXT SCREEN

Overall, how refreshed do you feel this morning?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! You will be asked to complete interviews throughout the day. Please do not turn off the phone.

Questions to be completed every 85 minutes

Displayed Instructions:

----------NEXT SCREEN

MOOD EXPERIENCES

----------NEXT SCREEN

Start the survey when you have 5 minutes without interruptions to complete all questions at once.

Think about how you were feeling when you STARTED the survey;

----------NEXT SCREEN

When you STARTED the survey how much were you feeling: (Same header for all questions below, take out "at the time of the beep"

Sad

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…

Angry

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…

Serene

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…

Relaxed

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…

Excited

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Happy

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Tired

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Pleasant

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Unpleasant

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Activated

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Calm

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Enthusiastic

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Worried

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…
Bored

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep…

Stressed

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

When you STARTED the survey how much were you feeling (use the same heading below):

Unable to control important things in life?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Confident about your ability to handle personal problems?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Things were going your way?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Difficulties were piling up so high that you couldn't overcome them?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Pain ANYWHERE?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Any urgency to urinate:

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Are you in a pain flare now:

I do not have a pain flare
I have a mild pain flare
I have a moderate pain flare
I have a severe pain flare

INTERVAL EXPERIENCES
SINCE YOUR LAST INTERVAL SURVEY

Did you feel any urgency to urinate?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Did you feel pain IN THE PELVIS?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Did you experience a FLARE in your pelvic pain?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Did you feel pain IN THE HEAD?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Did you feel pain ELSEWHERE IN THE BODY (Not the pelvis or head)?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

How much did pain anywhere interfere with yoru daily routine?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

SINCE YOUR LAST INERVAL SURVEY,
Did you have a void(s)?

No
Yes – recorded in event record
Yes – NOT recorded in event record

What were you doing AT THE TIME OF THE BEEP?
Describe _____

----------NEXT SCREEN

4 5

When did your most recent social interaction occur?

Less than 10 minutes ago
10-30 minutes ago
30-60 minutes ago
More than 60 minutes ago

_____(hh:mm) Need room for up to 10 entries

----------NEXT SCREEN

----------NEXT SCREEN

Who were you socializing?

CB family member?

With co-worker?

Subsequent questions conditional on yes to first one

With friend?

With stranger?

With other?

----------NEXT SCREEN

Was the interaction in person (1), by phone (2), or online (3)? ___

Was the interaction friendly?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Was the interaction hostile?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

At the time of the beep… What was your position?

(1) Standing
(2) Sitting
(3) Lying down

----------NEXT SCREEN

At the time of the beep… Describe your physical movement-

(1) None (Rest or Nap)
(2) Limited (Write)
(3) Light (Walk)
(4) Moderate (Jog)
(5) Heavy (Run)

----------NEXT SCREEN

At the time of the beep… Temperature comfort?

(1) Comfortable
(2) Too cold
(3) Too hot

----------NEXT SCREEN

At the time of the beep… Your location?

(1) Home
(2) Other's Home
(3) Work
(4) Vehicle
(5) Outside
(6) Other

1. RECORD INDIVIDUAL EVENTS EVENT: BATHROOM
    When did/will this activity occur? _____ AM/PM
    What type of activity are you recording (Check all that apply)
        Urination (ounces measured with "hat"_____)
        Bowel Movement (Description: Check all that apply)
            Hard, Normal, Loose, Painful, Straining

GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! Press the Exit button if you are satisfied with your responses. Reminder: Do not turn off the phone!

2. EVENT: ACTIVITY
    When did/will this activity occur? _____ AM/PM
    What type of activity are you recording (Check all that apply)
        Exercise (Mild, Moderate, Strenuous)
            How long did you exercise in minutes?
        Sex
        Stressful Experience
            Please describe the experience

GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! Press the Exit button if you are satisfied with your responses. Reminder: Do not turn off the phone!

3. EVENT: TOBACCO
    When did/will this activity occur? _____ AM/PM
    What type (Cigarette, Cigar, Chew, Other (Type))
        How many? 0 – 5+
4. EVENT: CONSUMPTION
    When did/will this acitivity occur? _____ AM/PM
    What type of consumption event?
        Meal (Meal type: Snack, Light, Moderate, Heavy)
        Caffeinated drink (What type: Tea, Coffee, Soda, Other)
            How much in ounces (needed for voiding diary)?
        Non-caffeinated non-alcoholic drink
            How much in ounces (needed for voiding diary)?

GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! Press the Exit button if you are satisfied with your responses. Reminder: Do not turn off the phone!

5. EVENT: ALCOHOL/DRUGS
    When did/will this activity occur? _____ AM/PM
    What type of event? (Check all that apply)
        Alcoholic drink (What type: Beer, Wine, Hard liquor)
            How much in ounces (needed for voiding diary)?
        Recreational drug
            Which one?

GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! Press the Exit button if you are satisfied with your responses. Reminder: Do not turn off the phone!

6. EVENT: MEDICATION
    When did/will this activity occur? _____ AM/PM
    What type of event? (Check all that apply)
        Allergy medication (Name : e.g. Benadryl, Zyrtec, Claritin, Allegra)
        Cold or cough decongestant (Name: e.g. Sudafed, Mucinex, Nyquil, Robitusin)
        Anti-inflammatory (Name: e.g. Ibuprofen, Naproxen, Advil, Alleve, Motrin)
        Non-study medications (Which one(s)?)
        Beta blocker or placebo (How many pills?)

GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! Press the Exit button if you are satisfied with your responses. Reminder: Do not turn off the phone!

7. EVENT: PAIN

When did/will this activity occur? _____ AM/PM
Pain Flare: How high would you rate the pain (0-10)?
Pain Flare: Location? (Check all the apply)
Pelvic
Body
Head
GREAT JOB! THANK YOU FOR COMPLETING THIS SURVEY! Press the Exit button if you are satisfied with your responses. Reminder: Do not turn off the phone!

ENGENDERS VOIDING DIARY COMPONENTS

Just prior to this void, did you have urgency (strong urge to urinate) …

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Just prior to this void, were you feeling pain IN THE PELVIS?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Just after this void, were you feeling pain IN THE PELVIS?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Pain Flare: How high would you rate the pain (0-10)?_____ Location check box (could be all of these): pelvic, body, head
Caffeinated drink: Radio buttons: Tea, Coffee, Soda, Other, how much in ounces (needed for voiding diary)
Alcoholic drink: Radio buttons Beer, Wine, Hard Liquor, how much in ounces (needed for voiding diary)
Non-alcoholic non-caffeinated drink: how much in ounces (needed for voiding diary)
Meal (radio buttons: snack, light, moderate, heavy)
Tobacco (Radio buttons: cigarette, cigar, chew, other_____; how many? 1-5)
Bowel Movement (Checkboxes: Hard, Normal, Loose, Painful, Straining)
Exercise (radio buttons mild moderate strenuous; how long in minutes_____?)
Sex
Stressful Experience (describe_____)
Allergy medication (eg benadryl, zyrtec, Claritin, allegra, etc.)
Cold or cough decongestant (eg Sudafed, mucinex, Nyquil, robitusin etc.)
Anti-inflammatory (eg ibuprofen, Naproxen, advil, alleve, motrin, etc.)
Recreational Drug (Which one? _____)
Other _____

Thanks, you are finished with this entry.

End of day items

----------NEXT SCREEN

On the following pages are descriptions of how you might have felt in the last 24 hours. Please select how you felt **in general in the last 24 hours**.

----------NEXT SCREEN

What type of day was today? (Check all that apply)

Regular work day
Short work day
Work from home
Non-work day
Sick day
Vacation day
Holiday
Other type of day (_____)

For the type of day you picked, was this day in any way unusual?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Please describe your day in a few words, focusing on what was different than a "typical day":

_____

----------NEXT SCREEN

Menstrual cycle questions:

If yes screen:

1) If you are having periods, when was the first day of your last menstrual period? (NA Free text vs Calendar depending on extent of programming)_____
2) This question conditional on not saying "NA" on last question –

Was this period painful?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours…

Did you engage in any kind of sexual activity?

(1) Yes
(0) No

If yes, what time did you start this activity? _____(hh:mm)

How many minutes did this activity last? _____

----------NEXT SCREEN

Were you feeling pain ANYWHERE over the last 24 hours?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Were you feeling pain in your PELVIC AREA over the last 24 hours?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Were you feeling pain OUTSIDE the pelvis over the last 24 hours?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Were you feeling urgency to urinate over the last 24 hours?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Which of the following physical symptoms did you experience over the last 24 hours?

----------NEXT SCREEN

Over the last 24 hours… **Headache?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Constipated/diarrhea?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Muscle soreness?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Tightness/pain in chest?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Chills?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Backache?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Cold/flu symptoms?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Joint pain?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Nausea/upset stomach?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Congestion?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Poor appetite?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Sore throat?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Over the last 24 hours… **Dizziness?**

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

----------NEXT SCREEN

Were there any unusually stressful events in the last 24 hours that you either missed in your survey entries or were noteworthy in their impact on your day? If so, please briefly describe it/them in the box below.

_____

----------NEXT SCREEN

Thanks, you are finished with this entry.

EVENT DRIVEN RECORD

Event driven items – for each one upon confirmation need to ask:

Event time: 5' from now; NOW, < 5' ago; 5-10' ago >10' ago;

ITEMS:

Urination (ounces measured with "hat"_____)

ENGENDERS VOIDING DIARY COMPONENTS

Just prior to this void, did you have urgency (strong urge to urinate) …

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Just prior to this void, were you feeling pain IN THE PELVIS?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Just after this void, were you feeling pain IN THE PELVIS?

Not at all	A Little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

Pain Flare: How high would you rate the pain (0-10)?_____ Location check box (could be all of these): pelvic, body, head
Caffeinated drink: Radio buttons: Tea, Coffee, Soda, Other, how much in ounces (needed for voiding diary)
Alcoholic drink: Radio buttons Beer, Wine, Hard Liquor, how much in ounces (needed for voiding diary)
Non-alcoholic non-caffeinated drink: how much in ounces (needed for voiding diary)
Meal (radio buttons: snack, light, moderate, heavy)
Tobacco (Radio buttons: cigarette, cigar, chew, other_____; how many? 1-5)
Bowel Movement (Checkboxes: Hard, Normal, Loose, Painful, Straining)
Exercise (radio buttons mild moderate strenuous; how long in minutes_____?)
Sex
Stressful Experience (describe_____)
Allergy medication (eg benadryl, zyrtec, Claritin, allegra, etc.)
Cold or cough decongestant (eg Sudafed, mucinex, Nyquil, robitusin etc.)
Anti-inflammatory (eg ibuprofen, Naproxen, advil, alleve, motrin, etc.)

Recreational Drug (Which one? _____)
Non-study Medications (Which ones? _____)
Beta-blocker or placebo (How many pills? _____)
Other _____

Appendix 3 ICECAN Data Collection Schedule (Appendix 3 Grid is stored separately)

ICECAN: Summary of required activities and evaluations according to time points of ascertainment

X	Required evaluations
X^p	Tasks and visits to be completed by CPP subjects
X^c	Tasks and visits to be completed by Healthy subjects
1	The informed consent must be signed BEFORE baseline evaluations are performed
2	All females of child-bearing age/capability must take a pregnancy test within 24 hours before enrollment
3	Questionnaires to be completed by subjects weekly at home
4	Each week subjects complete a weekly version at home
5	Subjects complete the full STAI questionnaire at baseline and complete only the State portion weekly and at each major visit
6	Valsalva maneuver is only performed at MCW
7	Subjects complete this once preferably the same day each week until Week 24 and the day before Visit 5
8	Subjects complete this on the same day as the ACP and 24-h HRV recording
9	Study physicians complete this at baseline, 12-week and 24-week visits.
10	Randomization takes place centrally at MCW
11	Blood draw is only performed at MCW and is optional
12	If SAE, that is severe and related scan and email to MCW within 24hr of knowledge of event or as early as possible
13	Healthy subjects complete study at Week 4 and then resume study at week 12 or 20 for another 4 weeks.