Chronic Pain Risk Associated With Menstrual Period Pain

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note extraneous material and internally relevant nomenclature has been edited from this publicly posted version

Today's date 7/20/2022

Study Protocol #9 (adult version): Deciphering the hormonal and nociceptive mechanisms underlying bladder pain DK100368

Public study title: Chronic Pain Risk Associated with Menstrual Period Pain

A. Specific Aims and Hypotheses:

Endometrial shedding during the menstrual cycle elicits profound changes in neuronal activity and cytokines producing moderate to severe pelvic pain in more than 20% of reproductive-age women.[1] Among these women, one out of every five develops chronic pelvic pain (**CPP)**. In contrast, women without dysmenorrhea rarely develop CPP. Furthermore, half of women reporting moderate-severe dysmenorrhea exhibit bladder pain at innocuous levels of bladder filling.[2] We have also shown that dysmenorrhea is a leading correlate of severe non-menstrual pelvic pain independent of psychological factors.[3] Identifying the mechanism responsible for the transition from dysmenorrhea to CPP could allow the development of preventative strategies.

We hypothesize the mechanism by which dysmenorrhea may lead to pelvic pain is cross-organ sensitization (**COS)**. The uterine inflammation during menstruation likely contributes to CPP by triggering neurogenic inflammation in adjacent organs.[4] This COS has been demonstrated with experimental injury and infection in animal models.[5,6] Our prior findings discussed above[2,3] motivate our present central hypothesis: *dysmenorrhea produces CPP via repetitive COS episodes*.

Although COS is believed to play a role in CPP, other studies of visceral pain[7,8] and PBS[9] have shown increases in visceral sensitivity involve impairments in descending inhibition, the normal counterbalancing outflow to pain-processing spinal neurons from the brainstem.[10] Also, since β-estradiol (E2) is elevated in dysmenorrhea[11–14], and increased E2 can worsen pain sensitivity[15,16], alterations in E2 could also contribute to increased prevalence of CPP in women with dysmenorrhea. A major limitation of prior studies is that they do not address the relationship between COS and impairments to descending inhibition. They also do not consider important covariates such as E2 levels and psychological factors (e.g., anxiety/depression).[17]

To determine the role of neurophysiological mechanisms in COS, while adjusting for these important covariates, we will characterize a novel phenotype of dysmenorrhea with bladder pain indicative of COS (D+COS). In our preliminary studies, women with the D+COS phenotype exhibited prolonged pelvic pain report in response to a mechanical vaginal stimulus. This hyperalgesia is common among CPP patients. However, in contrast to women with CPP, women with D+COS do not show significant psychological dysfunction. These findings imply dysmenorrhea, independent of psychological factors, influences vulnerability to pelvic pain. Additionally, our preliminary data and work by others show women with dysmenorrhea[18,19] have widespread reductions in pain thresholds, the hallmark of impaired descending inhibition also observed in IBS.[20,21] However, specific measures of descending inhibition have never been assessed in women with dysmenorrhea. To address this gap, we propose to conduct cross-sectional and prospective evaluations of pelvic nociception, reproductive hormones and psychological factors among women with dysmenorrhea. In this proposal, we will test the hypothesis that *repeated episodes of menstrual pain increase vulnerability to COS.*

COS may be reversible under ideal circumstances. Hormonal treatment of dysmenorrhea has already been shown to partially alleviate co-morbid irritable bowel syndrome (IBS) and

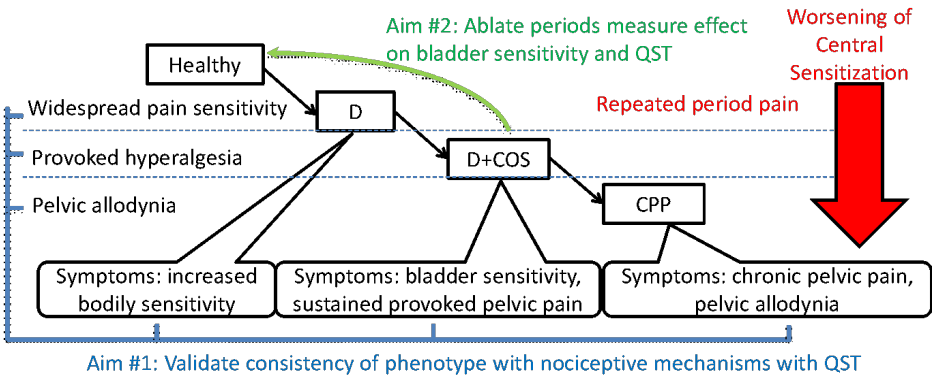


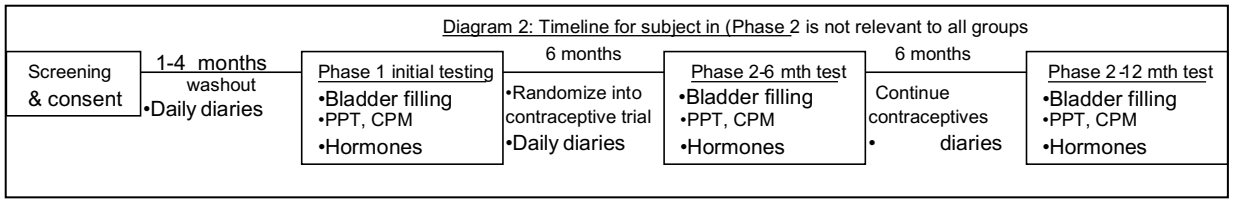
Diagram 1: We hypothesize repeated episodes of menstrual pain could impair descending inhibition of the spinal cord increasing widespread pain sensitivity. Additional local sensitization in the presence of worsening of descending inhibition may result in COS, provoked pelvic pain and bladder hyperalgesia. Elevated concentration of E2 in dysmenorrhea (not shown) could also increase bladder pain.

abdominal sensitivity[22], and improve painful bladder syndrome (PBS).[23] Our preliminary, cross-sectional data in dysmenorrhea sufferers suggest combined (estrogen and progestin) oral contraceptive (OC) users report less bladder pain at innocuous filling volumes. We would like to investigate further whether this is a modifiable phenotype. An equally critical issue involves identifying which patients would respond best to hormonal treatment. Previous studies have yielded conflicting results on whether OCs improve pain modulation.[24,25] These studies did not address dysmenorrhea or the usage of continuous OCs, which typically ablate withdrawal bleeding and withdrawal bleed-induced uterine nerve activation. *We hypothesize eliminating a painful stimulus with cyclical OCs will improve descending inhibition and reduce COS.* **(see Diagram 1)**

To determine whether alterations in pain are due to changes in descending inhibition, spinal cord excitability, or peripheral sensitivity we will use established tests for quantitative sensory testing. Changes in descending inhibition will be tested with conditioned pain modulation (**CPM**).[26] CPM involves presenting a counter-stimulus (such as cold water) to one part of the body and then simultaneously measuring the differential sensory response to a noxious stimulus elsewhere. The degree of descending inhibition is quantified by comparing the degree of blunted response from a counter-stimulus compared to the unmatched paradigm. Changes in spinal cord excitability will be tested with temporal summation.[27] Temporal summation involves evaluating pain report after repetitive stimulation of pain pathway sufficient to induce increased excitability of dorsal horn neurons. Changes in in peripheral sensitivity will be evaluated with mechanical pressure testing. We will perform these tests across multiple body sites to determine whether changes in pain mechanisms are regional or body-wide. Performing these tests will allow us to determine whether eliminating a painful stimulus with cyclical OCs improves descending inhibition, spinal excitability, or overall peripheral sensitivity, which will be done in the Phase 2 protocol. The addition of EEG and EMG recordings will also allow us to determine brain and spinal mechanisms that affect nociceptive modulation.

If OCs are shown to improve descending inhibition, our results will extend prior findings in animals that demonstrate descending inhibition is needed to maintain chronic pain states.[10] In humans, descending inhibition is impaired by repetitive pain insults[28,29] but normalizes with successful interventions.[30] Additionally, our results will establish a relationship between descending inhibition and COS allowing for the development of preventative strategies.[31]

Since endometriosis and fibroids are identifiable anatomical conditions but understanding of their mechanistic contribution to dysmenorrhea and bladder pain is limited, we will include confirmed endometriosis participants and fibroids participants mixed within our dysmenorrhea



cohort to determine which effects produced by the primary analysis are due to anatomical conditions.

Participants are divided into two arms. Arm #1 participants include women ages 18-45 with dysmenorrhea, women with painful bladder syndrome (PBS), and healthy controls. Arm #2 includes women ages 18-45 with chronic pain or special healthy controls.

Our hypotheses on mechanisms responsible for the transition of menstrual pain to bladder pain will be tested through the following phases:

Phase #1 (includes Arm #1 and Arm #2 participants): *To determine if dysmenorrhea with concomitant bladder pain sensitivity exhibits neurophysiological features consistent with established CPP.* Women with dysmenorrhea without COS and women with chronic pain conditions outside of the pelvic region will be used as controls. Quantitative sensory testing (**QST**) and a noninvasive bladder pain test we previously have validated will be used to determine whether impairments in descending inhibition and pelvic sensitivity are responsible for vulnerability to COS in women with dysmenorrhea (with and without COS) and/or CPP.

Phase #2 (includes a subset of Arm #1 participants only): *To differentiate the individual contributions of circulating sex hormones and repeated sensitizing events (painful menses) on descending and peripheral mechanisms of bladder pain***.** The same QST/bladder pain measures studied in Phase #1 will be retested within the D+COS and PBS subjects following a one-year randomized trial of cyclical vs. continuous OCs vs. no treatment. We added a second option for those who are randomized to Phase 2 that did not want to commit to their randomized group. They will be asked to come in for an additional assessment visit a year later. Participating in the randomized group is preferable but this will allow us to still obtain the follow up data that is important with these participants even if they choose not to participate in their assigned group.

Determining the neurophysiological relationship between repeated uterine inflammation, dysmenorrhea, and COS, and how OCs might reverse that influence, could define a new pathway for understanding the etiology of CPP/PBS. Secondly, identifying early markers of risk could ultimately underlie an effective prevention strategy because the onset of PBS is often gradual with limited symptoms.[32]

B. Research Design and Methods

B.1. Overview:

Diagram 2: After screening and washout, dysmenorrhea sufferers with and without COS, PBS patients, and chronic pain participants (with and without pelvic pain conditions) will be compared with controls on experimental pain sensitivity tests (**PPT**), which measure descending inhibition (**CPM**) and pelvic and bladder sensitivity (**Phase #1**). Women with dysmenorrhea with COS and PBS will be asked to participate in Phase #2. In Phase #2, a trial of cyclic and continuous OCs

will be used to differentiate the individual contributions of circulating sex hormones and repeated sensitizing events (painful menstrual periods) on descending and peripheral mechanisms of bladder pain. All participants will also complete a yearly follow-up questionnaire for 5 years (for participants who complete phase 2, the year 1 questionnaire will be incorporated into the 12-month testing Assessment #3 testing session).

This study will enroll a total of approximately 1,080 participants (See **Appendix 1**). Of those subjects, all will be enrolled at NorthShore University HealthSystem (NorthShore). This leaves a total of approximately 1,080 participants to be enrolled in arm #1 (1,020) and arm #2 (60) of the study. We anticipate that some arm 1 and arm 2 participants (up to 415 arm 1 and 10 arm 2 participants) may become ineligible after the screen visit and/or daily diary data because their pain levels do not clearly place them into a defined group, they are enrolled as a dysmenorrhea participant but do not show evidence of COS, medical reasons are identified at the screen visit that were not identified during the phone screen, or they become lost to follow-up between the screen and assessment visit. We expect approximately 645 arm 1 and arm 2 participants will continue the study and complete Phase 1; 515 will be women with dysmenorrhea, 40 will be women with PBS, 40 will be healthy women without pelvic pain or painful periods, 7 will be "special" healthy controls, and 43 will be women with chronic pain.

Chronic pain participants will be allowed to skip portions of the assessment based on their pain conditions and will be enrolled using drastically different inclusion/exclusion criteria compared to other groups. We anticipate approximately 88 women with either dysmenorrhea or PBS to continue participation in the longitudinal study and trial of oral contraceptives for Phase 2. We expect approximately 50 women who were randomized will decide not to participate in phase 2 but will choose to come back in a year later for an additional assessment visit for long term follow up data. Our ultimate goal is to prevent and reverse COS. We will ask all subjects to complete an at home yearly follow-up questionnaire for 5 years (for participants who complete phase 2, the year 1 questionnaire will be incorporated into the 12-month testing Assessment #3 testing session). If our hypotheses are correct about descending inhibition impairments being reversible with OCs, our work will spawn prevention studies for other highly co-morbid conditions associated with dysmenorrhea such as headache and fibromyalgia.

C1: Phase 1 Methods

C1.a Study Participants: Reproductive-age women (age 18-45) with: ARM 1: a) dysmenorrhea, b) dysmenorrhea and bladder sensitivity (D+COS), c) painful bladder syndrome (PBS), d) healthy, pain-free controls, ARM 2: e) 10 "special" healthy control participants, and f) 50 chronic pain female patients.

Participants will primarily be recruited from NorthShore's clinical sites in the Chicago-area. Participants may also be recruited from the community through advertising in NorthShore University HealthSystem's outpatient clinics; web-based advertising; posters and flyers in local businesses (coffee shops, athletic clubs) and on college campuses; referrals from local gynecologists; and the Illinois Women's Health Registry based at Northwestern University's Feinberg School of Medicine. All flyers and ads will be IRB approved. In our ads, we will call the study CRAMPP (Chronic Risk Associated with Menstrual Pelvic Pain). Participants may call our research hotline directly or after they indicate interest and give permission, their doctor will send us their contact information and a research coordinator will call the patient to formally assess eligibility using a phone screen. At the end of the phone screen, adult participants will be asked if we have permission to put their phone screen and contact information into our recruitment registry.

A research coordinator may periodically screen NorthShore's electronic medical records databases (EPIC) for patients within the NorthShore Medical Group system meeting eligibility criteria via a data warehouse request. This process will include the following:

1. Participant identification: Electronic databases will be queried for outpatient encounters using the ICD-9 codes associated with pelvic pain symptoms: endometriosis [617.x], uterine leiomyoma [218.x], dysmenorrhea [625.3], unspecified symptoms associated with pelvic organs [625.9], premenstrual tension syndromes [625.4], and other disorders of menstruation and abnormal bleeding from the female genital tract [626.8]. Bladder pain participants will be queried from: other symptoms involving the urinary system [788.99], dysuria [788.1], and interstitial cystitis [595.1].

2. The physicians of eligible women will be asked to ask the patient if they might be interested (via phone, letter, or in person during an appointment) and if we have permission to contact them (via phone, letter, or in person during an appointment). The physician will then notify us. Alternatively, the physician can just give the patient our contact information for THEM to call US if they are interested.

Only girls and women will be recruited because the study addresses dysmenorrhea and chronic pelvic pain, conditions unique to females. Notably, bladder pain syndrome also predominately affects women. Women above age 45 are excluded since dysmenorrhea only affects pre-menopausal women.

Inclusion/Exclusion Criteria for Healthy Controls, Dysmenorrhea, and PBS Participants (Arm 1)

Inclusion criteria: *Cases:* 1) **Dysmenorrhea** cases will have: a) average menstrual pain ≥ 5/10 (0=no pain and 10=the worst imaginable pain) with menses or withdrawal uterine bleeding from cyclic OCs without painkillers, b) menstrual pain in the region between the umbilicus and the perineum, above the level of the inguinal ligament and c) indication the participant has attempted to resolve pain by medical means (including NSAIDs and/or OCs).Participants who endorse bladder symptoms on the phone screen will be preferentially recruited; those who do not endorse bladder symptoms may be determined ineligible. We found our previous questions to have limited predictive value. Notably because we only want to collect the minimum amount of information necessary to determine eligibility on the phone screen, we may not ask all bladder questions (depending on what future statistics show in regards to what bladder questions accurately predict COS). Participants will complete a daily diary to verify menstrual pain during the screening period.[33] To be eligible as a dysmenorrhea case, a participant must indicate menstrual pain ≥ 5/10 on at least one day during menses in their daily diary. In the circumstance that a participant originally assigned to the dysmenorrhea group does not reach a pain of 5 on any days on the daily diary, this will be handled on a case-by-case basis particularly if they used a continuous around-the-clock, effective pain treatment regimen. They must have no concurrent chronic pain diagnoses that affect daily life or a history of more than 24 migraines per year. 2) **D+COS participants** will meet criteria for Dysmenorrhea cases, with >15 bladder pain on a 0-100 visual analogue scale (VAS) during either first sensation or first urge at assessment visit #1. Initially participants will be enrolled as dysmenorrhea participants and their final eligibility for D+COS will be determined after their assessment #1 visit. 3) Diagnosis of **PBS participants** will be confirmed by medical records indicating chronic (>3 months) pelvic pain (average intensity ≥ 3/10), pressure, or discomfort related to the bladder accompanied by at least one other urinary symptom such as persistent urge to void or frequency.[34] PBS

participants will also have records review to confirm the exclusion of other conditions by clinical examination or cystoscopy if necessary. PBS participants can have other chronic pain conditions.

Controls: 1) **Healthy control cases** will have a) average pain ≤ 3/10 with menses or with withdrawal uterine bleeding from cyclical OCs and b) no concurrent chronic pain diagnoses or ≥ 5 migraines in the past year. Healthy controls with < 2 migraines per year will be recruiting preferentially.

Exclusion criteria for dysmenorrhea (and D+COS), PBS and healthy control cases includes: a) presence of active pelvic or abdominal malignancies (primary or metastatic), b) absence of regular menses (including current pregnancy, recent pregnancy, or active breast feeding) c) active genitourinary infection in the last four weeks, d) unable to read or comprehend the informed consent in English, e) unwilling to complete study procedures f) presence of hypertension or risk for developing hypertension, and g) unwilling to withdraw from OCs for at least one cycle prior to the Phase #1 study visit. Participants with a history of epilepsy will not complete the visual unpleasantness task.

Inclusion/Exclusion Criteria for "Special" Healthy Controls and Chronic Pain Participants (Arm 2)

Special Healthy Controls (n=10) will be enrolled under the same inclusion and exclusion criteria as healthy controls, however, special healthy controls will not be required to have regular periods or abstain from hormonal contraception, and will not be assessed for migraines with aura.

Chronic pain cases (n=50) will have a diagnosed, documented (reviewed by PI) chronic pain disorder greater than six months' duration, which has required at least 2 different prescription treatments and/or surgical management. They must report an average pain of at least 5/10 in the last month. We will accept participants with any type of chronic pain (except for individuals with bilateral knee and hand pain) but anticipate many of our chronic pain participants to suffer from fibromyalgia, lower back pain, and chronic pelvic pain (including irritable bowel syndrome).

Of note, special healthy controls or chronic pain cases will not be excluded for unwillingness to undergo internal PPT testing, visual sensory testing, hip or head PPT. These groups may waive these procedures but still remain eligible to choose to participate in rest of the study.

Exclusion criteria for **Chronic pain cases (n=50) and Special Healthy Controls** includes a) presence of active pelvic or abdominal malignancies (primary or metastatic), b) pregnancy, c) active genitourinary infection in the last four weeks, d) unable to read or comprehend the informed consent in English, e) unwilling to undergo study procedures (except internal PPT testing, visual sensory testing, hip or head PPT which may be waived in these groups), f) bilateral hand or knee pain, or g) absence of ovaries.

To summarize, Chronic Pain Cases and Special Healthy Controls may have absence of regular menses, hypertension, a history of epilepsy (in which case sensory testing will not be performed) or migraines with aura, and be unwilling/unable to abstain from hormonal contraception.

Phone Screen

All callers will complete a phone screen to assess eligibility before being scheduled for a screen

visit. Because of the drastically different inclusion/exclusion criteria, separate phone screens will be used for arm 1 (healthy control, dysmenorrhea, and PBS participants) than for arm 2 (special healthy control and chronic pain participants. At the end of the phone screen, all callers who complete an arm #1 or arm #2 phone screen will be asked if we have their permission to keep their phone screen information and contact them for future studies. Participants who indicate yes will be added to our recruitment registry study upon study closeout. Eligible callers will be scheduled for a screening visit.

Note we may attempt to match groups for age and demographics. Thus, in some cases, eligibility on the phone screen may vary based on the study's current need for individuals with certain demographics for each group. We may also require dysmenorrhea participants to endorse bladder symptoms on the phone screen in order for them to be eligible to come in for a screen visit.

Procedures Completed During Each Study Visit: During each testing session, participants will have their blood pressure and pulse taken, urine collected, and a urine pregnancy test (unless no uterus) completed.

C1.b Screening Visit: Informed consent will be obtained by a trained research assistant before any questionnaires or study procedures are completed.

Adult Participants (arm #1 and arm #2) will complete the following questionnaires: a) complete medical, surgical, and gynecological history, and b) a psychosocial assessment using short-form PROMIS instruments (pain behavior, pain interference, fatigue, sleep, anxiety, depression, physical function, satisfaction with participation in social roles, and 10 item global health scale).[35] Participants will also complete the following pain rating questionnaires (the Interstitial Cystitis Problem and Symptom Indices, the Pain Catastrophizing Scale, Revised Short-form McGill Pain Inventory[72] and MIDAS headache questionnaire.)[67] These scales have been linked to pain intensity and quality of life in CPP sufferers, and catastrophizing (rumination, magnification, and feelings of helplessness about pain) is a well-validated intervention target for many cognitive-behavioral approaches for chronic pain.[36–39] Validated, CPP-specific assessment tools will be used to characterize IBS (Rome III criteria)[40] and PBS symptoms (Genitourinary Pain Index).[41] Subjects will also complete the Female Sexual Function Index (FSFI)[68] and BSI-18 based questionnaire containing 6 somatization questions selected from the BSI-18[69] plus a vision question, and the Complex Medical Symptoms Inventory (CMSI)-General, Fibromyalgia Symptoms, Fatigue, and Vulvodynia sections.[70,71] All questionnaires will be collected electronically using REDCap.

Note that midway through the study, an amendment was made for arm #1 and arm #2 study participants were freed from the requirement to undergo a complete gynecologic exam during the screen visit and as part of phase 1 participation. Instead, only arm #1 painful bladder syndrome and D+COS participants who randomize into phase 2 and agree to participate in phase 2 will be required to undergo a complete gynecological exam. These participants will be required to complete the gynecological exam at some point before their first phase 2 assessment visit (i.e. Assessment Visit #2 - 6 month visit). Transvaginal ultrasound and/or abdominal ultrasound may be performed on dysmenorrhea participants whose exam and/or history is suggestive of ovarian or uterine masses. Arm #1 PBS participants may choose to complete the gynecological exam during their screen visit if they prefer since their randomization to phase 2 is already determined.

Even though a gynecological exam is no longer required for phase #1, the second phase 1 visit

(assessment visit #1) includes internal vaginal pressure-pain threshold testing, which is similar in nature to the pelvic floor portion of a gynecological exam. Thus, for participants who have never had a gynecological exam, a study nurse or study doctor may complete a simple exam of the pelvic floor at the screen to promote understanding of the internal vaginal pressure-pain threshold testing that will be completed at the assessment. This ensures that the integrity of our data is not biased by unfamiliar experience. Participants will be asked whether they have ever had a gynecological exam on the phone screen and a simple exam of the pelvic floor for those that say they have never had an exam will be scheduled into their screen.

Participants entering phase 2 will still need to complete a birth control pill medical screen checklist in person or over the phone with a study doctor or study nurse before the pills can be prescribed.

Note arm #1 participants who indicate on a phone screen that they are unwilling to undergo gynecological exam will remain ineligible for the study since the assessment visit involves internal vaginal testing, which is similar in nature to an exam, and because our ultimate goal is to identify participants who will be eligible for phase 2, which requires a gynecological exam. However, participants enrolled in arm 2 with chronic pain who are unable to tolerate the internal testing may still skip this procedure.

Participants will be asked to do a tampon test originally designed to study vulvodynia.[42] The tampon test may be performed by the participant during the visit or at home. The tampon test is a standard test in which subjects report the amount of pain they feel on a 0-10 numerical rating scale after inserting a standardized tampon (Tampax original regular with plastic applicator, Proctor and Gamble).

Participants reporting average menstrual pain ≥ 5/10 on the phone screen (dysmenorrhea participants) will undergo a rapid version of the bladder test. Participants will be asked to rate their bladder pain on a 0-100 VAS scale when they reach “first urge.” If a participant arrives to the visit already at first urge, we will have them rate their pain and if not ≥ 10, we will wait 15 minutes past first urge and have them rate their pain again. This is because the participant may not have been focusing on the feeling of their bladder. Participants not already at first urge will be encouraged to drink water (at least 20 oz) during the screening visit until they reach first urge at which point a pain rating will be attained. After they reach first urge, they can go to the bathroom and leave their urine sample. Those with ≥ 10/100 pain at either first sensation or first urge and/or those with a GUPI scores ≥ 8 will be preferentially recruited into this study. In other words, we may eliminate some participants without evidence for COS (using the rapid bladder test FU pain score and possible using GUPI scores) after the screen visit. However, for those that continue, final group assignment will be determined by their ratings during assessment visit #1.

C1.c Between visit home tasks: Training of arm #1 and arm #2 participants to fill out daily diaries and how to use ovulation kits to determine their menstrual phase prior to their next visit will be provided by experienced research staff (for ovulation kits, only for those patients where menstrual phase changes can be defined). The daily diary will be completed using REDCap and asks them to rate their menstrual, bladder, and bowel pain (using a numeric rating scale where 0-no pain, 10-worst pain imaginable), describe any bleeding, enter results of ovulation tests, and list dose, type and use of painkillers. For special healthy controls and chronic pain participants (arm #2), the daily diary includes one additional pain rating where participants can choose the relevant area of pain from a scroll down menu and rate their pain in that area from 0-10. To protect confidentiality subjects will not enter identifying information and only use their assigned

subject ID # (see data collection section D). Dysmenorrhea, healthy control, and PBS participants will be asked to fill out daily diary entries for all days between their screening visit and assessment #1. Participants in the special healthy control and chronic pain groups will be asked to complete daily diaries starting the day after their screen visit and continuing for 35 days, regardless of when their assessment visit is scheduled.

C1.d Washout for arm #1 participants on OCs (not relevant for arm #2): Those on OCs at enrollment will complete a washout lasting a minimum of one menstrual flow in order to accurately confirm dysmenorrhea severity with daily diaries. Bleeding that occurs within 7 days of the cessation of birth control pills will not be counted as a menstrual flow, since this is the result of withdrawal bleeding rather than menstruation. Thus, most women currently taking birth control pills will need to abstain from taking hormonal contraception for at least 7 weeks to allow time for a withdrawal bleed followed by one full menstrual cycle (4 weeks) plus the time it takes them to enter their luteal phase (approximately 3 weeks). In the case that a woman does not experience withdrawal bleeding within 7 days of the cessation of birth control pills, determination of whether the bleeding that does eventually occur is the result of delayed withdrawal bleeding or menstruation will be made on a case-by-case basis. In the case that we cannot confirm group eligibility from one menstrual flow on the daily diary (i.e. they did not have great enough pain), we may ask the participant to continue to abstain from hormonal contraception for a second menstrual flow. If they are still not eligible after two menstrual cycles, they will be deemed ineligible or if applicable (pain is 3 or less on all days), switched to the healthy control group.

For the subset of participants who were using hormonal contraception to manage chronic or menstrual pelvic pain, and whom express concern about pain management during phase 1, the study doctor will discuss pain control options and if necessary, and the participant desires, prescribe Tylenol #3, Tramadol 50mg, or Norco 5/325. We expect these conditions to apply to a small subset of participants (less than 10). The participant may alternatively see their regular physician to address this issue. All other participants will be asked to manage their pain in the same ways they normally manage pain (e.g., over-the-counter painkillers, heat pads, etc.). All participants will be asked to indicate in their daily diary if they took any painkillers each day and if so, what medication and how much.

C1.e Assessment visit: Arm #1 and Arm #2 participants with regular periods and/or who are not using hormonal contraception will be asked to contact us when they start their first period after the assessment visit. The assessment visit will be scheduled during the anticipated participant's luteal phase (17-25 days after the start of their period adjusted if they typically have unusually short or long cycles). They also will be give ovulation kits and asked to complete them starting day 10 of their cycle up until their scheduled assessment visit to give us additional data about their menstrual phase. Bladder pain is less likely to be confounded with menstrual or ovulation pain during the luteal phase.

Special healthy controls and chronic pain participants (Arm 2) who do not have regular menstrual periods and/or are on hormonal contraception will not be required to use ovulation kits and instead visits will be scheduled at a time we do not expect any bleeding and approximately 2-6 weeks (but up to four months) after their screen visit.

The time between the screen visit and assessment visit may vary based on participant's personal schedule and ability to confirm ovulation status and group eligibility (if applicable) but will not exceed four months or be less than two weeks.

For arm #1 participants (healthy controls, dysmenorrhea, and PBS participants), group assignment is confirmed from one to four months of daily diaries. If the participant is not eligible for the originally assigned group after the first flow, we will ask the participant to complete the daily diary through a second menstrual flow to determine eligibility. If they are still not eligible for the originally assigned group after two flows, we will transfer the participant to the applicable group or inform the participant she is ineligible.

For arm #2 participants (Special healthy control and chronic pain participants) are asked to complete daily diaries starting one day after their screen visit and for 35 days, regardless of when their assessment visit is completed. For chronic pain participants, if as of two days before their scheduled assessment visit, their daily diary does not indicate consistent pain ratings, we may determine they are no longer eligible to complete the assessment visit. Healthy controls must demonstrate menstrual pain less than 4/10 and not indicate consistent pain ratings. Chronic pain or healthy control cases whose pain status is unclear will be handled on a case-by-case basis.

Assessment Visit #1 (Arm 1 and Arm 2)

Assessment Visits are very long so we will offer snacks to the participants (e.g., teddy grahams and fruit roll-ups).

During the assessment visit, EMG (of different muscle groups), EKG, and respiratory rate equipment will be applied and information will be recorded throughout the visit. EMG leads will be placed on the lower abdomen and right knee during the bladder testing to measure muscular recruitment. The abdominal EMG leads will be moved to the left knee once the bladder pain test is completed. The EKG leads and respiratory belt are placed to measure autonomic activity. The autonomic activity will be recorded to verify nociceptive experience.

Bladder pain test: All participants will first undergo bladder pain testing described in our validation study[2]. Subjects will be provided directions for the bladder study. The bladder study involves voiding and obtaining baseline measurements of bladder volume with a 3D ultrasound abdominal transducer (GE Voluson 750, Wauwatosa, WI). After hydration with 20 oz of water, subjects are instructed to report when they reach standard levels of bladder urgency: first sensation, first urge, and maximum tolerance and volumetric measurements of the bladder will be made. At each of these time points and every 15 minutes, 0-10 VAS measurements of pain and urgency are recorded. After scanning at maximum tolerance, subjects will urinate in a graded toilet hat to allow for measurement and saving a urinary specimen. Samples will be banked for analyses. If the participant gives permission in the consent form, urine samples will continue to be stored after the study is completed for possible future analyses by our lab on women's health conditions. If the participant does not give permission in the consent form for analyses beyond the current study, their sample will be destroyed upon study completion.

Questionnaires: The previous validated questionnaires from the screening visit will again be employed during the bladder testing. Participants will also complete a handedness questionnaire.

View of introitus in lithotomy position with relative position of pelvic floor exam points (exam finger inserts **into** introitus at these sites) (Lewis, 1918)

Cutaway of pelvis showing index finger examining left iliococcygeus site (#5) (adapted from (Peters, et al., 2007))

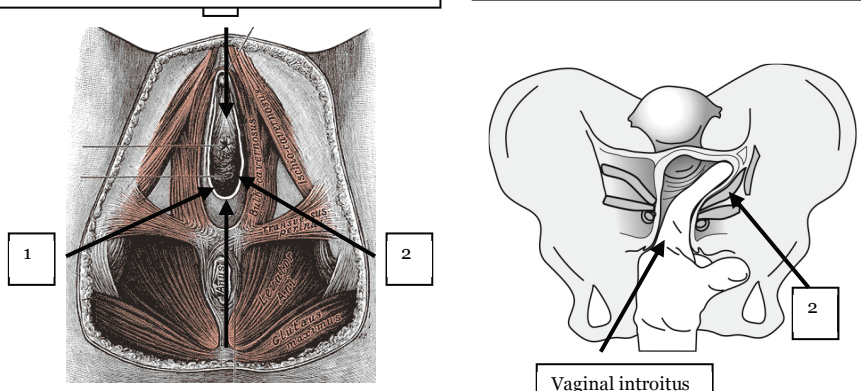


Diagram 3:
All points are examined 3 cm proximal to the vaginal introitus
#1: right iliococcygeus - located medial to the length of the tendinous arch
#2: left iliococcygeus
#3: 12 o'clock position, anterior vaginal wall (adjacent to the bladder)
#4: 6 o'clock position, posterior vaginal wall (adjacent to the rectum)

Pressure Pain Threshold Test:

Basic 8: Participants will undergo body and pelvic floor pressure pain threshold testing following the same validated protocols in our published studies[43,44] using digital external and internal vaginal pressure algometers. The body algometer (for example models see www.paintest.com) is a commonly used hand-held device in pain research that measures the amount of pressure applied to a 1 cm^2 circular rubber tip. The vaginal algometer consists of 2.5 cm^2 circular force sensitive resistor, which fits over the pad of the examiner's index finger under an exam glove (Detailed documentation was provided in a separate earlier supplement to the IRB). Prior to evaluation, subjects will report their baseline pain on an NRS 0-10 scale and will be asked to identify any painful body sites.

To evaluate bodily pain pressure thresholds, we will assess three external measurement sites corresponding to American College of Rheumatology fibromyalgia tender point sites: the right trapezius, the right medial knee fat pad, and the right greater trochanter.[45] One additional site will be a sham tender point, the middle of the forehead. A digital algometer (described above) will be used to conduct the external site measurements using a ramp rate of 0.5 $kg/cm^2/s$. Trials of the shoulder, hip, and knee may be terminated by examiner at 7.0 kg/cm^2 and trials of the forehead may be terminated at 4.0 kg/cm^2 in the case that the subject does not reach pressure pain threshold before then. Subjects will report level of pain at the time they pressed the button on a NRS 0-10 scale immediately after testing each individual site. After a two-minute break, the sites will be retested again.

Next, we will use the vaginal algometer (described above), The four pelvic sites will be accessed by intravaginal exam, with all measurements conducted using the right index finger

(Diagram 3) and a ramp rate of 0.5 kg/cm^2/s. Internal trials may be terminated by examiner at 3.0 kg/cm^2. A trained clinician will perform all pressure-pain tests. Subjects will report the pain at the time they pressed the button on a NRS scale immediately after testing each individual site. After a two-minute break, the sites will be retested again. In order to compare within-subject results from the three assessment visits and in order to standardize timing of post-testing time scores (collected every 5 minutes as described below), the order of testing sites will not be counterbalanced.

Subjects will be allowed to rest for another 5 minutes, and subject will be queried on the level of NRS pain at each site again (shoulder, hip, knee, forehead, global vagina). Subjects will be queried every 5 minutes until pain returns to baseline levels or up to 30 minutes (the time of resolution in minutes will also be recorded).

Conditioned Pain Modulation (CPM) : CPM testing involves repeating pressure pain threshold testing in the presence of a heterotopic stimulus (in this case cold water). The testing stimulus will be a mechanical pressure on the left medial knee fat pad. As in the first task, pressure will be applied (0.5 kg/cm^2/s ramp) until the participant achieves pressure pain threshold. Baseline pressure pain thresholds of the testing stimulus on the left knee and left shoulder will be determined. After waiting approximately 2 minutes, the conditioning stimulus will be applied by instructing the subject to insert their right hand up to their wrist into a bucket of water maintained between 0-6° C. After 10 s of immersion, subjects will be asked to rate their level of hand pain on a NRS 0-10 scale. After 20s of immersion, the pressure pain threshold will be re-applied using a 0.5 kg/cm^2/s ramp rate at the knee and the participant will be asked to rate their knee pain at the time they reached threshold (i.e. pressed the button) on a NRS 0-10 scale. After testing (~20s of immersion), subjects will immediately remove their hand and the pressure pain threshold will be determined at the shoulder. After waiting 5- and 10-minutes after water immersion, the testing stimuli will be re-applied. This paradigm combines a method for measuring CPM[46] and methods for accurately measuring participation of descending inhibition.[47] CPM will be measured by the standardized differences in pain threshold, EMG activity and autonomic responses before and after heterotopic stimulation. This strategy will provide the most reliable measurement of descending inhibition.[47,48]

Temporal Summation (TS): Participants will be instrumented to undergo tests of spinal pain modulation. Temporal summation will be tested using an algometer on the right medial knee fat pad. We will repeatedly apply an amount of pressure (0.5 kg/cm^2/s ramp rate; ITI = 5-15 seconds) equal to the average of the participant's two pressure pain thresholds assessed during the first task. After each press, the participant will be asked to rate their pain on an NRS 0-10 scale. The pressure will be applied ten times or until the participant rates their pain as a 6, whichever comes first.

EEG Testing: Subjects will be instrumented with a standard EEG cap. Encephalography (EEG) recordings will be obtained using a standard montage and equipment. A trained research assistant or nurse will place the EEG cap and electrodes. All participants will undergo a resting state EEG task in order to analyze brain activity patterns associated with pain behavior. Participants will be requested to focus on a fixation cross on a computer monitor with their eyes open and sitting with their eyes closed for one minute each. In total, the task is expected to take 10 minutes. All participants will complete a handedness questionnaire,[49] consisting of a series of questions asking which hand they use for different activities, in REDCap, which will help us interpret our EEG results.

Sensory Amplification Tests: To evaluate whether sensitivity extends to other modalities we will use a software application that measures self-reported unpleasantness to increasingly loud and bright stimuli (safe and well below thresholds that could cause auditory or visual damage).

Auditory stimuli will be provided by waveforms generated by a PC and transmitted through headphones calibrated by an audio monitor[50] . Each stimulus will be a two-tone combination of 1200 and 1350 Hz. Sounds will ranged in 5 dB steps from 35 to 90 dB SPL (A-weighted). After receiving instructions, the subject will be presented with a series of sounds, in random order of intensity. After each 2 s stimulus, the participant will be asked to rate its unpleasantness from 0 ("not at all unpleasant") to 20 ("the most unpleasant sound imaginable").

Visual stimuli will be displayed on a computer monitor capable of providing up to 300 lux. In this 3 min task, two visual stimuli will be presented in an alternating block design. The control stimulus is a fixed crosshair centered in the middle of a solid background; the experimental stimulus will be a flashing (12.5 Hz) blue/yellow checkerboard presented at varying levels of brightness (luminance) in both an ascending and random manner. Each subject will be presented with 6 test patterns alternated with 6 blanks patterns. Subjects will use a 0 ("not at all unpleasant") to 20 ("the most unpleasant pattern imaginable") numerical descriptor scale to rate the unpleasantness of each visual stimulus and the entire task.

The visual task may be annoying or elicit a headache or nausea in sensitive individuals. Participants may stop at any time.

The sensory testing will take in total about 20 minutes and will be performed while the participant is connected to EEG.

Additional quality controls: We may need to repeat a task, modify electrode positions, reconfigure equipment, or adjust features in the environment to evaluate the quality of our data acquisition scheme. For example, if a PPT measurement was not obtained or an electrode does not stay put, we may ask the subject to allow us to repeat additional measurements.
All implanted quality controls with permission from the subject will be documented in the subject record.

C1.f Hormone Measurements with Blood (Arm 1 and Arm2): We will ascertain that subjects have not had more than 100 ml of blood drawn over the past 24 hours or 200 ml over the past 30 days. After completion of the pressure/pain testing, a trained nurse or physician will draw up to 60 mL of blood into vacutainers. Separation of serum and plasma will be performed using centrifugation and samples will be aliquoted and banked appropriately to allow for hormone measurements and analyses. To control for the potential influence of reproductive hormones on pain sensitivity, and test our hypothesis about the role of hormonal influences on neurophysiological pain assessments, we will measure serum samples for E2, P4 and testosterone using methods that do not cross-react to the OCs from core laboratories.[51] If the participant gives permission in the consent form, blood samples may also be analyzed for genetics. If the participant gives permission in the consent form, blood samples will continue to be stored after the study is completed for possible future analyses by our lab on women's health conditions. If the participant does not give permission in the consent form for analyses beyond the current study, their sample will be destroyed upon study completion.

C1.g Follow-up questionnaires: We will ask arm 1 and arm 2 participants to complete an annual follow-up questionnaire including the validated questionnaire instruments mentioned above and a general medical history questionnaire. All arm 1 and arm 2 questionnaires will be

collected electronically using REDCap at home. If requested, paper versions can be provided instead and mailed in. For participants continuing to Phase 2, the year 1 annual medical history questionnaire will usually take place during their assessment #3 visit. All participants that complete phase 1 (Screen + Assessment #1), including phase 2 dropouts, will be asked to complete the annual follow-up questionnaire.

Group (Sample Size)	**H (20)**	**D (20)**	**D+COS (95)**	**PBS (40)**	**P (50)**	**MSA**
Bladder Pain at FU (VAS)	0	0	2.5	3.0	-	0.85
Pelvic Hyperalgesia (VAS)	0	0	3	3	-	0.84
Pelvic Hyperalgesia (Time)	0	0	2	2.5	-	0.83
Pelvic Pain Thresholds	0	.5	.5	2.3	-	0.81
Bodily Pain Thresholds	0	1.5	2	2	2.3	0.91
Conditioned Pain Modulation	0	*1-2*	*1-2*	4_a	$2\text{-}4_b$	0.94
Menstrual Pain History	0	3.7	5	6	-	0.81
Temporal Summation	0	0	*.7*	*.8*	-	-
Estradiol	0	$1\text{-}3_c$	$1\text{-}3_c$	*1-3*	-	0.84
Depression/Anxiety	0	.3	.4	2	2-4	0.82

Table X: Sample sizes and hypothesized results in Z-scores or range of Z-scores (relative to healthy) for subjects that are healthy (H), have dysmenorrhea (D), have cross organ sensitization (D+COS), PBS, or chronic pain (P). MSA indicates Kaiser's measure of sampling adequacy. Numbers in italics indicated minimum detectable hypothesized score with current sample size. Z-scores based on preliminary data, or others indicated by subscript (a: [9], b:,c:[11,13]). Effects temporal summation in these populations are unknown, but effect sizes as large as 1.1 have been reported for fibromyalgia. Dashes (-) indicate unknown effect size.

C1.h Data Analysis/Sample Size Requirements: The primary outcomes under this hypothesis will be [1] bladder pain at FU, [2] pelvic hyperalgesia (VAS, time to return to baseline pain), reflecting peripheral dysfunction/sensitization, and [3] centrally mediated hyperalgesia (CPM, bodily pain thresholds).

Factor [1], Bladder pain at FU: Our preliminary data on healthy subjects and women with dysmenorrhea, in combination with other studies investigating migraine and PBS, suggests effect sizes between 1.4-5.6 (Cohen's d). As a control comparison, we expect that women with chronic pain will have levels of bladder and pelvic sensitivity comparable to healthy subjects. We will perform t-tests for each of these three pain variables using pairwise contrasts and appropriate Bonferroni corrections for six group comparisons.

Factor [2], pelvic hyperalgesia: To confirm pelvic hyperalgesia (*but not necessarily pelvic PPT*) is higher in those with bladder pain, we will show bladder pain and pelvic hyperalgesia (both VAS and TIME) are higher in D+COS than healthy pain-free controls (H), dysmenorrhea only (D), and chronic pain patients (P). Similarly bladder pain, pelvic hyperalgesia and pelvic floor pain sensitivity (PPT) are anticipated to be greater in PBS than H, and D.

Factor [3], centrally mediated hyperalgesia: Greater central dysfunction manifest by impaired CPM and reduced bodily pain thresholds in vulnerable subjects will be shown by comparing D+COS, PBS, and P to D and H. We estimate 15 subjects per group will provide adequate power (g-power, a priori t-test, α=0.05, β=0.2). To verify we have adequate phenotyping of CPM, we have included the positive control group with chronic pain (P). We assumed the

difference in CPM between healthy subjects and chronic pain patients in this protocol would have an effect size of 0.78 based on a meta-analysis (Lewis et al. 2012). Using a 2.6 allocation ratio, a priori power analysis (α=0.05, β=0.2), suggests we need 20 healthy participants and as many as 50 chronic pain patients to detect a difference in the mean CPM. Additionally, the utilization of 50 chronic pain patients will allow us to use regression to identify up to 5 predicted factors associated CPM with correlation coefficients exceeding 0.3 even after correcting for multiple comparisons (α=0.05, β=0.2) as suggested by an a priori random model power analysis. This will be readily captured as our global study design encompasses a larger sample size (Table X) to handle anticipated secondary analysis and projected needs for completion of Phase #2.

Additional confirmatory analysis: Special healthy control subjects (n=10), we will verify special healthy control subjects do not differ from original enrolled healthy control subjects (n=20) to ascertain chronic subjects (P) participating in the trial are not treated differently under blinded conditions.

For the secondary analysis of Phase #1, we will evaluate the D+COS (provoked bladder pain) phenotype while accounting for menstrual history (including historical OC exposure), hormonal factors, anxiety, and depression. To determine the relative contribution of these secondary variables on bladder pain, we will perform principal component analysis (PCA). We have adequately powered the analysis of these potential covariates consistent with a bootstrap (n=1000 replications, Fig Y) model based on our preliminary data and studies by others (described in Table X). Even with weak correlations (r=.3), PCA can detect 3 primary factors with our projected sample size. PCA is an optimal method to identify the mechanisms responsible for bladder pain. Historically PCA with even smaller sample sizes has been employed to measure how coping skills influence pain perception during the menstrual cycle[52] (n=20) and to show that different pain testing modalities are related to differential psychosocial profiles[53] (n=188). Even if PCA fails, we can make many direct comparisons beyond what is described here with t-tests.

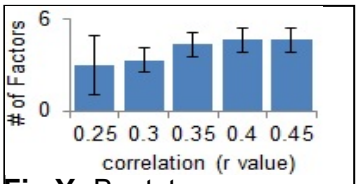


Fig Y: Bootstrap simulation constructed assuming a range of correlations between potential mechanisms (e.g. pelvic hyperalgesia and bladder pain) indicate that even with weak correlations we can detect 3 factors. Error bars show 95% confidence intervals.

C1.i Expected outcomes: *(1) Abnormal CPM is the primary predictor of widespread pain sensitivity observed in dysmenorrhea, D+COS and PBS.* Results would be insightful if CPM was worse in participants with D+COS and PBS than those with dysmenorrhea alone because this would indicate CPM mechanisms are involved in COS. On the other hand, if CPM impairments are universal across chronic pain patients, PBS, D, and D+COS compared to healthy controls, this would indicate that pain independent of location and chronicity depends on impaired descending inhibition. This finding would be the basis for developing a physiological biomarker for those at risk of developing CPP/PBS similar to approaches by others identifying risk for chronic pain[45,79]. On the other hand, some pain conditions such as rheumatoid arthritis[114] and trapezius myalgia[115] do not seem to exhibit CPM. The vulnerability to conditions unrelated to CPM may involve either inflammatory or specific peripheral nerve events. If CPM does not correlate with widespread pain sensitivity or bladder pain phenotypes in this proposal, we have adequately powered our sample size to detect alternative mechanisms that could be responsible (e.g. menstrual, hormonal, psychological). Nevertheless, consistent with meta-analyses on the menstrual cycle[54], psychological[55] , and central factors on pain[56], we anticipate central pain factors to explain the majority of the variance in bladder pain sensitivity. Determining the relative importance of systemic E2 concentrations vs. other modifiable factors such as

menstrual pain will provide insight on the most important mechanisms for reducing bladder pain sensitivity in vulnerable women.

(2) Pelvic hyperalgesia and severity of menstrual pain are more common in D+COS phenotype vs. dysmenorrhea only. The identification of clinical biomarkers such as severity of pain after a speculum exam (analogous to the post pelvic floor PPT pain report) can be obtained from our results. Our study results would provide evidence of the significance of this routine clinical exam in reliably predicting bladder sensitivity.

C1.j Potential Problems: *Primary and/or secondary comparisons are nonsignificant: a mixture of mechanisms are involved in D+COS phenotype or PBS.* We will confirm whether our paradigm was sufficient to detect deficits in CPM using our positive control group with chronic pain to validate a true negative finding. Afterwards, we will use cluster analyses to identify phenotype patterns and confirm mechanistic differences with banked serum. *Why not examine additional hormones?* We are examining hormones that have been frequently studied in pain research and will bank serum allowing for the future possibility of studying others. *What about endometriosis, fibroids and other known anatomic uterine pain conditions?* To account for the common conditions (especially within our medical practice), we will include 10 confirmed endometriosis (operative report review) participants and 10 fibroids participants (ultrasound or surgical confirmation) mixed within our dysmenorrhea cohort to determine which effects produced by the primary analysis are due to anatomical conditions.

C2. Methods for Phase #2

C2.a Participation criteria

All phase #1 participants with D+COS (pool of 95 candidates) or PBS (pool of 40) will be enrolled with intent to transition into phase #2, though the second phase of the study will be presented as optional. If they choose not to participate in their randomized group, they will have the option to come back in a year later for an additional assessment visit for long term follow up data. These enrollment targets, accounting for a potential 35% dropout rate, will enroll 62 participants with D+COS and 26 PBS. This will provide adequate power for the prespecified contrasts.

Inclusion Criteria: 1) Participation in Phase #1 and assigned to the Arm #1 PBS or D+COS group, 2) willing to comply with OC assignment (continuous for PBS; cyclical, continuous, or no OC for D+COS).

Exclusion Criteria: a) presence of active pelvic or abdominal malignancies (primary or metastatic), b) absence of regular menses (including current pregnancy, recent pregnancy, or active breast feeding), c) unwilling to undergo pelvic examination/testing, e) standard contraindications to use of OCs:

- Thrombophlebitis or thromboembolic disorders, or a history thereof
- Cerebrovascular or coronary heart disease
- Known or suspected carcinoma of the breast
- Endometrial cancer or other known or suspected estrogen-dependent neoplasia
- Undiagnosed abnormal genital bleeding
- Jaundice with prior pill use
- Hepatic adenomas or carcinomas
- Known or suspected pregnancy
- Hypersensitivity to any component
- BMI >40

- Lactation (< 6 weeks postpartum)
- Age >35 years and smokes 15 cigarettes/day
- Elevated blood pressure (160 / 100)
- Chest pain (angina pectoris)
- Vascular disease
- Major surgery with prolonged immobilization
- Complicated valvular heart disease
- Migraine with focal neurologic symptoms
- Diabetes with evidence of nephropathy, retinopathy, neuropathy, vascular disease, or > 20 years duration
- Severe (decompensated) cirrhosis

Women with persistent, nonphysiological ovarian cysts or with a fibroid larger than 5 cm will be excluded because of associated bleeding problems and likelihood to undergo surgery during participation. Women taking drugs listed on the manufacturer's web site (such as penicillin and topiramate) that may reduce OC effectiveness will be instructed to use condoms as a backup form of contraception.

C2.b Randomization: NorthShore's biostatistical core will assign D+COS participants to continuous OC, cyclical OC, or the untreated arm using a permuted-block randomization scheme with block sizes only known by appropriate study staff (PBS as above will be only assigned to continuous OCs) adjusting for the unequal recruitment goals (n=10,26,26) . To the extent possible, the research nurse performing QST will be blinded to group assignment. Because of withdrawal bleeding in the cyclical group, we cannot blind participants to their assignment. Drs. Tu and Senapati will be responsible for prescribing both cyclical or continuous OC usage and will assess the participant for contra-indications and answer questions at the screen visit. The study team will provide counseling that will emphasize the benefits and risks of OCs as well as the lack of protection against sexually transmitted diseases from OCs and options for backup contraceptive methods. Participants will be instructed to begin OCs the Sunday immediately after commencing their menses or on a day that allows for personal choice. We will give all participants a "Tips for use of Oral Contraceptives" flyer.

C2.c Trial of OC usage: Participants in Phase #2 of the study will be prescribed generic microgestin (20 µg ethinyl estradiol/1 mg norethindrone acetate), a lower estrogen-containing OC well-tolerated by women. Microgestin has minimal breakthrough bleeding compared to a continuous OC regimen with higher estrogen concentration or different progestin[57] or a cyclic paradigm with lower estrogen concentration.[58] D+COS participants will be randomized into three study arms: cyclical OC usage (n=26) vs. continuous usage (n=26) vs. no OC (n=10). Less untreated subjects are needed as large effect sizes are expected compared to OC users. Cyclical OC users will be instructed to take 21 days active followed by 7 days of no pills every four weeks. Continuous OC users will be instructed to immediately start the next pack of active pills on day 22. Those not taking OCs will be instructed to use barrier contraception. At the time of randomization, participants will be counseled by the study team on the proper usage of these treatments. Participants assigned to OCs will receive a three or four month prescription for microgestin and have it filled at the Evanston Hospital Outpatient Pharmacy. All refills will be distributed by the same pharmacy. Separately from the D+COS randomization scheme, PBS participants will receive only continuous OC. This study design deliberately avoids randomizing chronically symptomatic PBS patients onto cyclical vs. continuous OCs, as the anticipated difference in outcomes is small, and therefore not likely to be favorable in recruiting subjects. However, identifying which pain mechanisms (visceral and pelvic sensitivity) change following OC-induced hormonal suppression both in chronically symptomatic PBS sufferers vs. the

asymptomatic D+COS patients may prove crucial to understand which PBS patients exhibit hormonally responsive pain.[23]

C2.d Study Visits Participants in Phase #2 will return to repeat experimental pain and sensory assessments identical to the initial Phase #1 and complete the questionnaires referred to in the screening visit plus a side effects questionnaire at six months and one year after OC initiation (which follows a two month washout in Phase #1). These visits should occur on pill days (not cycle days) 7-21 of the cyclical group and any day for the continuous group, which is comparable to cycle days 15-28 in Phase #1. Control subjects not taking OCs will participate during their post-luteal phase days 15-28 (similar to Phase #1). This will make hormonal levels comparable and minimize any vaginal bleeding. For those who are randomized to Phase 2 but decides to not participate in their randomized group, they may be asked to come in a year later for an additional assessment visit to obtain long term follow up data. These participants will be asked to complete daily diaries and ovulation kits for one month prior to the annual assessment visit, similar to Phase 1.

C2.e Home Assessments: Participants in Arm #2 will complete the daily diary during the fifth and eleventh month of OC administration[33,60]. They will also complete a monthly questionnaire asking questions related to their pain, mental health, and any side effects they may be experiencing (comprised of the McGill Pain Inventory, a side-effects questionnaires, and PROMIS anxiety item bank, PROMIS depression item bank, and PROMIS pain interference item bank). They will not need to do these questionnaires during months 6 and 12 at home because they will do them during their study visit for assessments #2 and #3 respectively. When done at home, they should complete these assessments on or near day 15 of their pill pack for participants on cyclical OCP, day 15 of their menstrual cycle (15 days after their menstrual period started) for participants not taking OCPs, and simply 28 days apart for participants taking continuous OCP.

C2.f Compliance: To evaluate compliance, participants will be asked to return all used and partially used OCP pill packs.

C2.g Potential Adverse Events: *Breakthrough bleeding:* Participants in each arm will be counseled about uncommon risks of breakthrough uterine bleeding. Participants experiencing abnormal bleeding episodes will be offered visits with the study gynecologists, Drs. Tu and Senapati, to rule out cervical or vaginal sources of bleeding. Women who need management of breakthrough bleeding will be managed per standard clinical protocols. Those needing to terminate OCs will also be withdrawn per standard protocols. Withdrawn participants will still be asked to complete the annual follow-up questionnaire and may be asked to still do the 6- and 12-month visit as intent-to-treat and as a separate comparison group. Women will be instructed to contact a health care provider right away for numbness or severe headaches which are not tension headaches and are not relieved with NSAIDS, severe leg pain, severe chest pain, shortness of breath, blurred vision, flashing lights, or blindness, or severe abdominal pain.

C2.h Data Analysis/Sample Size Requirements: To complete Phase 2, we propose analyses assessing a) the effect of concurrent and historical OC exposure on experimental bladder pain, b) whether continuous and cyclical OC regimens differ in effect on bladder pain sensitivity (via elimination of priming by menstrual pain events), and c) the effect of OCs on nociceptive mechanisms (pelvic hyperalgesia, conditioned pain modulation). The anticipated effect sizes with required sample sizes (which we meet/exceed) are listed in Table Z.

	ES	n_{req}	n_{pre}	n_{post}
Z1	f=.7	10	95	62
Z2	dz=.6	24	80	52
Z3	dz=.6	24	40	26
Z4	d=.8	52	80	52
Z5	d=1.6	16	80	52

Table z: Planned comparisons with lowest anticipated effect size (**ES**). Required n_{req} estimated with α=0.05, β=0.2. Total enrolled D+COS and/or PBS subjects per analysis are shown before (n_{pre}) and after attrition (n_{post}) as detailed in III.d.

(Z1) We will establish prospectively whether usage of cyclic or continuous OCs affects bladder pain sensitivity (on bladder filling test), in women with D+COS compared to women not on OCs. We are adequately powered to detect improvements in bladder pain, assuming a 35% dropout rate (conservative estimate, well exceeding previously published rate of 18-24%[51,59]) and accommodating a 1:5.2 ratio of non-OC to OC users. We will perform repeated measures ANOVA to determine if experimental bladder pain improves at 6 months compared to baseline in women with D+COS (all having washed out at enrollment). Follow-up results at 12 months will be used to confirm findings. The effect size (f=0.9) was estimated from our preliminary data above. Analyses will be adjusted with E2 and P4 levels as a covariate to determine if the effect of OCs on bladder pain is largely due to its effects on circulating hormones with standard methods[60].
(**Z2)** Similarly, we will establish whether among D+COS patients' pelvic hyperalgesia and descending modulation, the hypothesized mechanisms responsible for bladder pain sensitivity, are affected by OC usage. We will perform a repeated measures ANOVA to determine whether significant improvements occur in pelvic hyperalgesia (VAS pain report, and time to normalize), pelvic PPT threshold, bodily PPT thresholds, temporal summation, and CPM in OC treated vs. untreated subjects at 6 and 12 months. Cohen's d's for drugs that affect PPT[61], temporal summation[62] and CPM[63] range between 0.6-0.9.

(Z3) Similar to z1, we will determine if bladder pain sensitivity improves among PBS patients pre- and post-continuous OC exposure using repeated measures ANOVA (6 and 12 months). Although hormonal treatment has been shown to improve bladder pain in 13/15 patients with PBS[23], the exact effect size is not known. Prospectively OC usage has been shown to reduce episodes of urinary calculosis pain (d=0.8)[64]. We are adequately powered to detect an effect size as low as dz=0.6.

(Z4) We will perform ANOVA to determine whether continuous OC usage is superior to cyclic usage for reducing bladder sensitivity, absolute levels of E2/P4, and dysmenorrhea in women with D+COS (6 and 12 mths). Legro and colleagues have established superiority of continuous OC usage over cyclic usage for dysmenorrhea (d=1.6) using a comparable sample size. After surgical removal of endometriosis, continuous OC usage is associated with less non-menstrual pelvic pain than cyclic OC usage (d=0.8)[65]. Our published data[2,3] suggests a strong relationship between dysmenorrhea and bladder pain. Therefore, we predict a difference of d=0.8 on bladder sensitivity between the groups. If differences are significant, we will evaluate whether any specific nociceptive mechanisms are different between cyclical and continuous OC users by comparing the effects of QST, temporal summation and CPM between groups.

(Z5) To determine whether menstrual, hormonal, central or peripheral changes are most responsible for improvement or worsening of bladder pain, we will use a repeated measures design. We will compare menstrual history, pelvic hyperalgesia, pelvic floor PPTs, external PPTs, CPM, E2/P4 concentrations in D+COS subjects that have >50% improvement in experimental bladder pain to those that have <50% improvement (6 and 12 mths). A similar type analysis showed the effect of duloxetine on CPM in improving diabetic neuropathy (n=30).[31] If mechanistic differences are comparable to Yarnitsky (d=1.6), we are more than adequately powered with 62 subjects with D+COS accounting for attrition. To confirm the proposed mechanism we will perform multi-level factor analysis. We performed a bootstrap analysis with our hypothesized data set (n=1000 repetitions) and confirmed we can measure 2 independent components (each consisting of 2-5 factors) with 95% certainty (median Kaiser Sampling Adequacy =0.75).

C2.i Expected outcome: We anticipate analyses (z1-z5) will show OC usage reduces bladder pain primarily by restoring descending inhibition via extinguishing menstrual pain. This finding would be consistent with preliminary observations in IBS and PBS.[9,22,23,59,66] Peripheral sensitivity and circulating hormonal concentrations are expected to have a limited effect as a covariate (z2,3,5). Analysis z4 is expected to show the continuous OC usage is superior to cyclic OC usage for improvement of PBS, in consensus with studies by others on dysmenorrhea and COS.[22,51] While we have observed a strong relationship between dysmenorrhea and bladder pain[2,3], accomplishment of Phase #2 will provide prospective evidence in a randomized controlled trial that bladder pain sensitivity is a modifiable risk factor and understanding of the mechanisms responsible. They will be mailed the ovulation kits to complete before their 6- and 12-month visits.

C2.j Potential Problems: *Differences in OC management within each group:* There will likely be differences in treatments within groups because some participants will require additional OC pills to reduce bleeding, and PBS subjects often rotate medications to treat pain flares. To account for confounding, all deviations will be documented and accounted for as a covariate. We previously confirmed the success of this strategy in our cross-sectional study showing that women on OCs have less bladder sensitivity (see section IV.c.1). Also, women who stop taking the birth control pills but continue as intent-to-treat will not receive their monthly questionnaires on day 15 of each cycle (since presumably discontinuing the pills would shift their cycle). They will continue to receive the monthly questionnaire every 28 days as they would have had they continued the pills.

D. Data Collection and Storage:
Confidentiality: data will be collected by our study coordinator, who has already undergone human subjects protection training and has experience with data handling through our pilot studies. All questionnaire data and data forms will be maintained in REDCap and exported into excel sheets onto a password-protected server as needed for analysis. *The only paper forms used in this study will be the consent and W-9 forms (for payment) containing subject name (no ID), and a study overview checklist, oral contraceptive eligibility checklist, session notes form containing the subject ID only (no name).* Hard copies will be kept in a locked office or cabinet. Electronic data will be coded only with a unique study identifier for participants, linked to a single master list. The phone screen will also be maintained in REDCap and contain PHI. PHI is necessary to collect in order to create an Epic medical record for research participants. At the conclusion of the study, patient identifiers on any hard copies will be blacked out in marker on all data forms.

Quality assurance: All questionnaires and data forms will be entered using REDCap.
Questionnaire data will be reviewed at the time of each evaluation in REDCap by our research coordinator to ensure all questions are answered and coded appropriately. For pressure-pain threshold, the data acquisition program will automatically save these values. Data will be reviewed and backed up onto the shared drive after each session. Computer parameters will identify potential outliers (using a two standard deviation cutoff) and illogical data, which will be reviewed by the principal investigator. Differences will be reconciled using the session notes form, which will be used to record any applicable extra information which may affect testing (e.g., temperature of room, participant very tired) or any errors that occurred during a session.

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