

1 **Appendix S1. Detailed Methods**

2 *Abuse screening*

3 To identify abuse history or exposures among patients, we utilized the University of North
4 Carolina sexual and physical abuse questionnaire. This instrument has been used extensively in studies
5 of IBS patients; we used a slightly modified version that has been used in pelvic pain studies.¹ The
6 instrument has demonstrated acceptable reliability and validity in female gastroenterology clinic
7 patients.

8 *Clinical exam and pain threshold detailed protocol*

9 A palpometer (described below) was used to calibrate exam palpation pressure to 0.4-0.6
10 kg/cm². Vaginal tissue compliance, voluntary pelvic floor contractility, and pelvic floor gross muscle
11 strength were quantified on exam using Likert scales. Flexibility of the vaginal introitus was measured
12 from 0-3 (0 = vaginal caliber <1 finger, 1 = able to fit 1 finger, 2 = able to fit 2 fingers, and 3 = able to fit 2
13 fingers with room to wiggle). The ability of the pelvic floor muscles to relax after contraction was scored
14 0-2 (0 = 100% relaxation after contraction, 1 = some relaxation, and 2 = 0% relaxation). General pelvic
15 floor muscle strength was assessed with vaginal exam during a voluntary pelvic floor contraction
16 (between 0 = no palpable muscle contraction up to a score of 5 = strong muscle contraction).

17 *External PPTs:*

18 Pressure was applied to four external sites (shoulder, forehead, hip and knee) at a rate between
19 0.5 and 1.0 kg/cm²/s using a pain pressure algometer similar to the internal algometer described in our
20 prior publications.² Participants squeezed a trigger at the moment the pressure turned to pain at which
21 each trial was terminated. For safety, if the participant had not yet pressed the button by the time the
22 examiner reached 4.0 kg/cm² on the forehead or 7.0 kg/cm² on the shoulder, hip, or knee, the test was
23 terminated. For analytical purposes, in the few subjects who exceeded the safety threshold without

24 reporting pain, the cutoff value was used instead. After the four sites were completed, the participant
25 was given a five minute break and the four sites were repeated a second time.

26 *Internal Pelvic PPTs:*

27 Pressure was next applied to four transvaginal pelvic floor sites (right and left iliococcygeus, anterior
28 bladder, and posterior anorectal raphe) at a rate between 0.5 and 1.0 kg/cm²/s using a fingertip
29 mounted algometer. The examiner terminated each trial when the participant indicated pressure had
30 turned to pain by again squeezing a hand-held trigger. For safety, all testing terminated at 3.5 kg/cm²,
31 regardless of participant response. To avoid learning effects, we randomized the order in which sites
32 were tested. After the four sites were complete, the participant was given a five-minute break and the
33 four sites were retested. The average threshold across trials was used for final analyses. Both post-PPT
34 pain and duration were recorded to evaluate the safety and comfort of PPT testing and to characterize
35 sustained hyperalgesia (hereafter referred to as *aftersensation pain*).³

36 *Bladder testing extended details*

37 We used detailed descriptors for a prior study to operationalize the ICS' published cystometric
38 thresholds.⁴ Definitions were as follows: 1) first sensation – When riding in a car, the driver pulls over to
39 a rest stop to urinate, you would go as well, 2) first desire to void- When riding in a car, you would
40 initiate the request to find a rest stop to urinate, 3) maximal capacity- When riding in a car, you would
41 urinate on the side of the road in bumper to bumper traffic.

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