Diarrhoea and Constipation Link to Faster Accumulation of Heat Pain in Women

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This thesis is presented in partial fulfilment of the requirements for the degree of Bachelor of Sciences (Honours), Murdoch University, 2019.

Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary educational institution.

Ye Di

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Abstract

The cause of chronic abdominal pain in irritable bowel syndrome remains unclear. This study linked gut microbiome (GM; a recently proposed cause) with pain modulation (an established cause). GM composition was inferred by stool consistency (measured on the Bristol Stool Scale). Pain modulation was tested using the conditioned pain modulation paradigm, where a "pain-inhibits-pain" phenomenon was examined. Participants were 24 women with or without irritable bowel syndrome. First, women with diarrhoea or constipation (reflecting imbalanced GM composition) and women with normal stools showed no pain inhibition to electrical stimuli after heat conditioning. Lack of pain inhibition in both groups may be due to inappropriate study design (e.g., short delay after preliminary pain sensitivity tests and mildly painful stimuli). Second, women with diarrhoea or constipation had a faster accumulation of pain when their forearm was heated than women with normal stool consistency. This finding suggests a link between imbalanced GM composition and heightened pain facilitation. However, the results must be interpreted cautiously because of confounds, such as stimulus intensity and unpleasantness level. Major implications for future studies are (a) to develop a standardised conditioned pain modulation testing protocol, (b) to measure distress or anxiety levels during the testing, (c) to measure the unpleasantness of painful stimuli together with pain intensity, (d) to use an accurate measure of GM composition, and (e) to replicate the findings in large sample.

Keywords: gut microbiome, stool consistency, pain modulation

SSDiarrhoea and Constipation Link to

Faster Accumulation of Heat Pain in Women

Chronic abdominal pain is the main symptom of irritable bowel syndrome (IBS), a bowel disorder affecting around 11% of the population globally (Canavan, West, & Card, 2014). Chronic abdominal pain has a long-lasting negative impact on both physical and mental quality of life (Litleskare et al., 2019). Treatment, however, has limited effectiveness because its cause remains unclear (Holtmann, Ford, & Talley, 2016). This study aimed to bridge a gap between an established cause (dysfunctional pain modulation) with a recently proposed cause (gut microbiome composition) to understand chronic abdominal pain in IBS. Results can facilitate future effective treatment.

Dysfunctional Pain Modulation in IBS

An established cause for chronic abdominal pain is dysfunctional pain modulation from the brain, which leads to an increased sensitivity to pain signals (Farmer & Aziz, 2009). Visceral pain signals are transmitted to the brain by spinal sensory neurons (Mayer & Raybould, 1990). The brain can inhibit this pain transmission, a process called diffuse noxious inhibitory control (DNIC) in animals or conditioned pain modulation (CPM) in humans (Kwon, Altin, Duenas, & Alev, 2014). Pain modulation includes pain inhibition and pain facilitation; an imbalance between these influences is implicated in several chronic pain conditions (Goubert, Danneels, Graven-Nielsen, Descheemaeker, & Meeus, 2017; Petersen et al., 2017). As for chronic abdominal pain, when pain facilitation outweighs pain inhibition, the transmission of visceral pain signals increases, which makes a person prone to perceive abdominal pain. As such, dysfunctional pain modulation plays a part in IBS.

Pain inhibition is commonly evaluated by the CPM paradigm, which tests a “pain-inhibits-pain” phenomenon (Honigman, Yarnitsky, Sprecher, & Weissman-Fogel, 2013). This phenomenon happens when pain caused by a painful stimulus, applied to a remote part of the body (the conditioning stimulus), inhibits pain caused by another painful stimulus (the test stimulus). Successful inhibition of the pain caused by the test stimulus indicates functional pain inhibition (Honigman et al., 2013). On the other hand, pain facilitation can be indicated by the accumulation of pain during prolonged conditioning, known as the temporal summation of pain (Horn-Hofmann, Kunz, Madden, Schnabel, & Lautenbacher, 2018; Wilder-Smith & Robert-Yap, 2007).

In an early study that applied the CPM paradigm, women with IBS showed decreased pain inhibition and increased pain facilitation when compared with healthy women (Wilder-Smith & Robert-Yap, 2007). Furthermore, these changes in pain modulation varied across IBS subtypes. According to stool consistency, there are diarrhoea-predominant IBS (IBS-D) and constipation-predominant IBS (IBS-C; Lacy et al., 2016). Women with IBS-D had less pain inhibition and more pain facilitation than those with IBS-C (Wilder-Smith & Robert-Yap, 2007). These findings may explain more severe abdominal pain reported by people with IBS-D (Canavan et al., 2014). In later studies applying the CPM paradigm, researchers confirmed decreased pain inhibition in IBS and found that women with IBS were sensitive to a wide range of painful stimuli (Piche, Arsenault, Poitras, Rainville, & Bouin, 2010; Piche, Bouin, Arsenault, Poitras, & Rainville, 2011; Piche et al., 2013; Zhou, Fillingim, Riley, Malarkey, & Verne, 2010). Specifically, women with IBS had increased pain sensitivity to electrical, heat, and cold stimuli across their upper and lower limbs. Moreover, in recent meta-analyses, people (mostly women) with IBS had nearly five-fold less pain inhibition than healthy people (Albusoda et al., 2018;

Marcuzzi et al., 2019). The results from these meta-analyses also suggested a link between more severe IBS symptoms and worse pain inhibition (Albusoda et al., 2018; Marcuzzi et al., 2019). Therefore, factors that affect the function of pain modulation may play a part in and serve as a therapeutic target for chronic abdominal pain.

Imbalanced Gut Microbiome Composition in IBS

In the past decade, an increasing amount of evidence suggested a gut-brain interaction. The gut-brain interaction is a bidirectional relationship between the enteric nervous system (the gut) and the central nervous system (the brain; Guo, Chen, Xing, & Liu, 2019; Martin, Osadchiy, Kalani, & Mayer, 2018). Consequently, functional bowel disorders were renamed “disorders of gut-brain interaction” (Lacy et al., 2016). The gut microbiome (GM) and its metabolic products then become a potential cause for functional bowel disorders including IBS (Herrera, Robles-Alonso, & Guarner, 2016; Rodino-Janeiro, Vicario, Alonso-Cotoner, Pascua-Garcia, & Santos, 2018). The GM refers to micro-organism living in the human gut, which enjoys a mutual relationship with the host (De Preter & Verbeke, 2016). For example, the GM absorbs nutrients from food consumed by the host and protects the host from pathogens (De Preter & Verbeke, 2016). Healthy people have a rich, diverse, and stable GM composition, which is harmed in the case of dysbiosis (Stecher, Maier, & Hardt, 2013). Dysbiosis refers to an imbalanced GM composition, which occurs through different ways: overgrowth or disappearance of GM species, changes in the amount of GM species, or genetic mutation of GM species (Stecher et al., 2013). Because people with other chronic pain conditions experience dysbiosis (Minerbi et al., 2019), there may be a link between imbalanced GM composition and chronic abdominal pain in IBS.

There are differences in GM composition between healthy people and people with IBS, within subtypes of IBS, and among severity of IBS (Liu et al., 2017; Pozuelo et al., 2015; Tap et

al., 2017). Specifically, when compared with healthy people, people with IBS have less *Bifidobacterium* and *Lactobacillus*, which are well-known probiotics (Liu et al., 2017). Probiotics are one kind of GM that, when given in adequate amounts, improve GM composition and contribute to the host's health (Hill et al., 2014). Within IBS subtypes, people with IBS-D have fewer probiotics in their gut than those with IBS-C (Liu et al., 2017). In detail, the GM composition of people with IBS-D is characterised by reduced diversity and unusual dominance of GM species such as *Bacteroidetes*, which links to increased abdominal pain (Jalanka-Tuovinen et al., 2014; Pozuelo et al., 2015). In contrast, the GM composition of people with IBS-C has fewer differences when compared with healthy people (Pozuelo et al., 2015). That means, people with IBS-D have more severe dysbiosis than those with IBS-C, which, again, may explain more severe abdominal pain in people with IBS-D. In short, more severe dysbiosis may relate to more severe abdominal pain.

Both animal and human studies support this speculation. In a review of animal studies, imbalanced GM composition increased abdominal pain sensitivity, and treatment by probiotics decreased such sensitivity (Mayer, Tillisch, & Gupta, 2015). In another review on animal studies, after transplanting an imbalanced GM composition (stool sample) from people with IBS to animals, the recipient animals exhibited an increased abdominal pain sensitivity (Collins, 2014). As for human studies, in a meta-analysis of 53 randomised placebo-controlled studies, therapeutic interventions that targeted GM composition had a limited but significant beneficial effect for abdominal pain in IBS (Ford, Harris, Lacy, Quigley, & Moayyedi, 2018). Two recent randomised, double-blind, placebo-controlled studies, which were not included in Ford et al.'s (2018) meta-analysis, found the same beneficial effect (Hod et al., 2018; Jadresin et al., 2017). Specifically, in both studies, improving imbalanced GM composition reduced the intensity of

abdominal pain for people with IBS. To sum up, the research reviewed above highlights a potential link between imbalanced GM composition and chronic abdominal pain.

Evidence for a Link Between GM Composition and Pain Modulation

Healthy GM Composition May Increase Pain Inhibition

A healthy GM composition may improve pain inhibition by changing brain connectivity. Several brain regions play a role in pain sensitivity, including the prefrontal cortex (PFC), the insular cortex, and the periaqueductal grey matter (PAG) in the midbrain (Kwon et al., 2014). The PFC and the insular cortex are active during an acute pain experience; however, they are much more active in people with IBS than healthy people (Lowen et al., 2015). The PFC and the insular cortex send signals to the PAG, which then sends downstream signals to the brainstem to generate a pain inhibitory influence (Kwon et al., 2014). The extent to which the PAG communicates with the PFC and the insular cortex predicts pain sensitivity. In detail, greater resting-state connectivity between the PAG and the PFC predicts decreased pain sensitivity (Z. Li et al., 2016; Mainero, Boshyan, & Hadjikhani, 2011). In contrast, greater resting-state connectivity between the PAG and the insular cortex predicts increased pain sensitivity (Ploner, Lee, Wiech, Bingel, & Tracey, 2010).

In a study conducted in healthy women, promoting a healthy GM composition increased connectivity between the PAG and the PFC and decreased connectivity between the PAG and the insular cortex (Tillisch et al., 2013). In other words, a healthy GM composition generates positive changes in brain signalling centred on the PAG that increases pain inhibitory influences. As a result, these positive changes decrease pain sensitivity and improve abdominal pain.

Healthy GM Composition May Decrease Pain Facilitation

A healthy GM composition may reduce the strength of pain facilitation by altering cellular communication in the gut. The brain modulates pain transmission by using serotonin (5-HT), one kind of neurotransmitters (Kwon et al., 2014). Around 95% of 5-HT in the human body is produced by the enterochromaffin cells in the gut via a particular enzyme, tryptophan hydroxylase 1 (Gershon, 2013). Metabolic products of GM, short-chain fatty acids, increase the amount of this enzyme, and so increase the production of 5-HT (Reigstad et al., 2015). Short-chain fatty acids also increase 5-HT release from the enterochromaffin cells. The enterochromaffin cells have a particular receptor, olfactory receptor 588, that responds to short-chain fatty acids (Bellono et al., 2017). One sort of short-chain fatty acids, isovalerate, activates this receptor, resulting in 5-HT release (Bellono et al., 2017). The release of 5-HT, however, activates 5-HT^3 receptors on mucosal sensory neurons to facilitate rather than to inhibit pain transmission (Bellono et al., 2017; Kwon et al., 2014). That means, a large amount of short-chain fatty acids, probably due to an overgrowth of GM species (a trigger for dysbiosis; Stecher et al., 2013), increases the amount of 5-HT in the gut, which then facilitates the transmission of abdominal pain signals. These processes may explain why increased 5-HT release linked to increased abdominal pain both in people with IBS and healthy people (Cremon et al., 2011; Keszthelyi et al., 2015; Zhao et al., 2015).

From the above, inhibiting 5-HT^3 receptors (to decrease pain facilitation) may indirectly improve abdominal pain. Indeed, alosetron, a compound that inhibits 5-HT^3 receptors, is effective in treating abdominal pain in people with IBS (Black et al., 2019; Camilleri et al., 1999). Another way is to promote the effectiveness of 5-HT reuptake, where 5-HT transporters take 5-HT back to the cell and inactivate it (Gershon, 2013). In recent animal studies, probiotics

promoted the expression of 5-HT transporters in the colon of a rat model of IBS, leading to a reduction of colonic 5-HT volume (Cao et al., 2018; H. Li, Wang, Huang, Li, & Zhang, 2019). Although these findings come from animal models, it is tempting to speculate that a heathy GM composition may decrease pain facilitation and improve abdominal pain.

Research Aim and Hypotheses

In summary, the available evidence suggests that people with IBS experience chronic abdominal pain and have imbalanced GM composition and dysfunctional pain modulation. A healthy GM composition may improve the function of pain modulation, which then improves chronic abdominal pain. Hence, an imbalanced GM composition may interfere with pain modulation through the gut-brain interaction, leading to increased abdominal pain sensitivity. As such, this study aimed to examine the link between GM composition and pain modulation to understand chronic abdominal pain better and to facilitate future effective treatment. Before stating the hypotheses, the following sections outline the choice of study design.

Inferring GM Composition by Stool Consistency

Generally, GM composition is examined by advanced stool analysis (e.g., Navas-Molina et al., 2013). Nevertheless, it is also possible to infer GM composition based on stool consistency (a more feasible option in the present study). The Bristol Stool Scale (BSS) classifies stools into seven types (Saad et al., 2010). Lower scores (Types 1 and 2) indicate constipation with harder stool consistency. Higher scores (Types 6 and 7) indicate diarrhoea with looser stool consistency. These extreme BSS scores suggest an imbalanced GM composition (Tigchelaar et al., 2016; Vandeputte et al., 2016). In contrast, middle BSS scores (Types 3, 4, and 5) are considered normal stool patterns, suggesting a healthy, balanced GM composition (Saad et al., 2010; Tigchelaar et al., 2016; Vandeputte et al., 2016).

In studies that applied the BSS in people with IBS-D, participants' abdominal pain score decreased as their BSS scores decreased to the middle scores (Brenner et al., 2019; Hod et al., 2018; Whitehead, Duffy, Sharpe, Nabata, & Bruce, 2017). Similarly, in healthy participants, pain sensitivity on their hands decreased as their BSS scores decreased to the middle scores (Shiro, Arai, Ikemoto, & Hayashi, 2017). These results suggested that the link between GM composition and pain modulation may exist both in people with IBS-D and healthy people with looser stool consistency.

Likewise, in a study applying the BSS in people with IBS-C, participants' abdominal pain score decreased as their BSS scores increased to the middle scores (Schmulson et al., 2019). Moreover, in a study conducted in people with chronic pain, a positive link was found between pain severity and constipation severity (Arai et al., 2018). In other words, the results of Arai et al.'s (2018) study suggested that participants' pain symptoms became worse when their BSS scores departed from the middle scores to the lower end. Hence, by using the BSS, one could expect that these extreme BSS scores (Types 1, 2, 6, and 7) would link to abdominal pain severity and so to pain modulation.

Assessing Pain Modulation by the CPM Paradigm

As noted earlier, pain inhibition is evaluated by the CPM paradigm: Pain caused by the conditioning stimulus inhibits pain caused by the test stimulus (Honigman et al., 2013). Pain facilitation can be indicated by the speed of accumulation of pain during prolonged conditioning (Horn-Hofmann et al., 2018; Wilder-Smith & Robert-Yap, 2007). Because people with IBS have pain hypersensitivity to heat and electrical stimuli (Piche et al., 2010), heat and electrical stimuli were used as the conditioning and the test stimuli, respectively, in the current study. Because the pain hypersensitivity exists across different body sites, including forearms (Piche et al., 2010),

both stimuli were applied to the ventral forearms. Given that standardised painful stimuli may be undetectable to some participants yet be unbearable to others (King et al., 2009), heat and electrical stimuli were delivered with personalised pain intensity. Using personalised pain intensity minimised the risk of overlooking important effects during testing due to undetectable or unbearable pain.

Generally, in studies that applied the CPM paradigm, researchers measured pain intensity to the pre-conditioning test stimulus, then applied the conditioning and the test stimuli at the same time, and measured pain intensity to the test stimulus again (e.g., King et al., 2009). They then examined if the pain to the test stimulus during conditioning is lower than the pain to the pre-conditioning test stimulus. According to the recommendations on the CPM testing (Yarnitsky et al., 2015), applying the test stimulus immediately after (as post-conditioning), rather than in parallel, helps minimise distraction due to an overlap of two sensations. Thus, the suggested sequence was used in the present study: the pre-conditioning test stimulus (electrical), the conditioning stimulus (heat), and the post-conditioning test stimulus (electrical). If pain to the post-conditioning electrical stimulus is less intense than pain to the pre-conditioning electrical stimulus, it suggests functional pain inhibition. When examining changes in pain intensity to heat stimulus during prolonged heat conditioning, it indicates the speed of accumulation of pain (implying pain facilitation).

After heat conditioning, there was a recovery period, consisting of repeated post-conditioning electrical stimuli. This additional measure was inspired by the findings that healthy women had a rebound of pain to the test stimulus after removing the conditioning stimulus, whereas women with IBS had no change in pain intensity across time (Bouhassira et al., 2013; Piche et al., 2010; Piche et al., 2011; Piche et al., 2013). In other words, in healthy women,

decreased pain intensity to the post-conditioning test stimulus (due to functional pain inhibition) "recovered" to previous pain intensity to the pre-conditioning test stimulus after removing the conditioning stimulus. In contrast, women with IBS had no decrease in pain intensity to the post-conditioning test stimulus (due to dysfunctional pain inhibition) so that their pain reports remained the same across time. In brief, including the recovery period aided in examining pain inhibition.

Summary and Hypotheses

In summary, this study was designed to examine a link between extreme BSS scores (reflecting imbalanced GM composition) and dysfunctional pain modulation (tested using the CPM paradigm). Evidence suggests that this link may exist both in people with IBS and healthy people with diarrhoea or constipation. Given that women are almost three-fold likely than men to have IBS (Canavan et al., 2014), this study examined women with or without IBS. It was hypothesised that women with extreme BSS scores (Types 1, 2, 6, and 7) would have less pain inhibition than healthy women with middle BSS scores (Types 3, 4, and 5). It was also hypothesised that women with extreme BSS scores would have more pain facilitation than healthy women with middle BSS scores.

Method

Research Design

The main design was a repeated-measures design. The dependent variables were pain intensity to electrical and heat stimuli reported by participants. There were three independent variables (IVs). First, based on stool consistency measured on the BSS, participants were assigned to two Group (IV 1 [IBS, control]), which represented different GM compositions. The IBS group included women with IBS and healthy women with extreme BSS scores (Types 1, 2,

6, and 7). Specifically, healthy women who had more than 25% of extreme BSS scores out of total BSS scores went to the IBS group. This 25% threshold is based on the criteria for IBS subtype classification (Longstreth et al., 2006; see Appendix A for details). The control participants were healthy women who had less than 25% of extreme BSS scores. Second, changes in seven pain reports to electrical stimuli (E) delivered across Time (IV 2 [E1, E2, E3, E4, E5, E6, E7]) indicated the function of pain inhibition. Third, changes in five pain reports to heat stimuli (H) collected every minute during 5-min Heating (IV 3 [H1, H2, H3, H4, H5]) indicated the strength of pain facilitation.

Participants

Participants were 29 women (IBS, n = 13; control, n = 16) recruited through the flyer (Appendix B). Participants completed the initial safety screen questionnaire (Appendix C) and signed the informed consent form (Appendix D) before the procedure. Exclusion criteria were pregnancy, breastfeeding, chronic pain, any medical or mental conditions, and any treatments for these conditions. Three control participants withdrew from the study because they could not tolerate the heat pain. One IBS participant who took pain medications was excluded. One control participant was also excluded, given her reporting high pain intensity to heat (deemed an outlier based on the boxplot examination). Thus, the data analyses included 12 IBS and 12 control participants. In the IBS group, five women had a past diagnosis of IBS and reported abdominal pain. This study was approved by the Murdoch University Human Research Ethics Committee (2019/040; Appendix E).

Materials and Apparatus

BSS diary. A stool diary (Appendix F) was designed for participants to record their stool type daily based on the BSS (Figure 1, the next page). The BSS is a 7-point ordinal scale that

indicates stool consistency (Saad et al., 2010). The BSS classifies stools into seven types. Types 1 and 2 indicate hard and dry stool consistency (constipation); Types 6 and 7 indicate loose and watery stool consistency (diarrhoea); and Types 3, 4, and 5 are considered normal stools (Saad et al., 2010). The BSS has 81% overall accuracy in classifying stool types and 76% overall inter-rater reliability (Blake, Raker, & Whelan, 2016). In this BSS diary, participants gave a BSS score for every bowel movement if more than one defecation within a day. Participants stated "no bowel movement" if no defecation within a day. Participants reported their weight, height, and menstrual cycle. Participants also reported if they were diagnosed with IBS (and the year of diagnosis) and if they experienced abdominal pain during the recording.

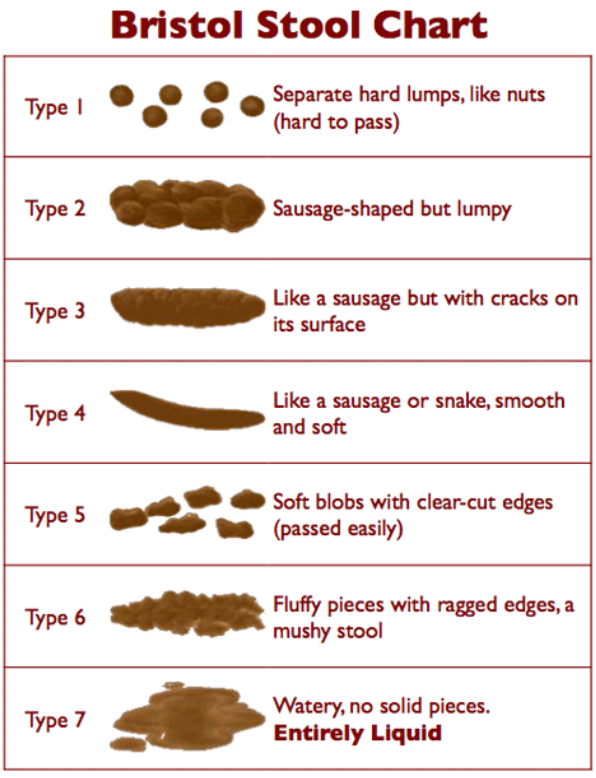

Figure 1. The Bristol Stool Scale (BSS) used in the stool diary for participants to record their stool patterns. Types 1 and 2 indicate constipation; Types 6 and 7 indicate diarrhoea; and Types 3, 4, and 5 are normal stool patterns. Adapted from Saad et al. (2010, p. 404).

Pain Catastrophizing Scale. Anxiety about pain influences pain reports (Quartana, Campbell, & Edwards, 2009). Hence, the Pain Catastrophizing Scale (PCS; Sullivan, Bishop, & Pivik, 1995) was used to examine the impact of pain anxiety on pain perception. The PCS is a reliable (total coefficient alpha = .93) and valid measure of pain anxiety (Osman et al., 1997). It includes three subscales (rumination, magnification, and helplessness), which are significant predictors of the intensity of physical and emotional distress during pain experience. The PCS score was obtained by averaging scores of three subscales.

Verbal pain rating scale. Participants reported pain intensity on an 11-point verbal pain rating scale (Appendix G), which ranges from 0 to 10. In this scale, 0 indicates *no pain*, 1 indicates *the presence of pain*, 4 indicates *mild pain*, and 10 indicates *extreme pain*.

Electrical stimulus (the test stimulus). A constant current stimulator (DS7A; Digitimer, Welwyn Garden City, United Kingdom) was used to generate an electrical pulse (4-pulse train with 0.5 ms pulse duration and an inter-pulse interval of 5 ms). The electrical stimuli were delivered via a custom-built concentric electrode. It consisted of a copper wire cathode centred within a ring-shaped stainless-steel anode with an inner diameter of 10 mm and an outer diameter of 20 mm. The electrode was attached to the participant's left or right forearm.

Heat stimulus (the conditioning stimulus). A custom-built thermal probe with a 10-mm diameter was used to deliver heat stimuli. The heat stimuli were delivered to the participant's forearm contralateral to the arm that received the electrical stimuli.

Procedure

During the initial screening, the participant read the information letter (Appendix H), completed the initial safety screen questionnaire, and signed the informed consent form. She

then started recording her stool types from Day 1 to Day 14 (recording length was suggested by Lacy et al., 2016) and attended the testing session on Day 15.

On the day of testing, to minimise potential effects of alcohol, nicotine, caffeine, and exercise on pain perception (Horn-Hofmann, Capito, Wolstein, & Lautenbacher, 2019; Kosiba, Zale, & Ditre, 2018; Lemley, Hunter, & Bement, 2015; Overstreet, Penn, Cable, Aroke, & Goodin, 2018; Thompson, Oram, Correll, Tsermentseli, & Stubbs, 2017; Vaegter, Handberg, Jørgensen, Kinly, & Graven-Nielsen, 2015), the participant was asked to refrain from taking the substances and from exercising at least 2 hr before testing. After the participant arrived at the laboratory, she read the information letter, signed the informed consent form, and completed the PCS. Two student researchers conducted the following procedure (see Appendix I for participant instructions).

Preparing forearm test sites. The participant's ventral forearms were cleaned with a pumice stone, rinsed with water, and dried with tissue paper. It was to minimise skin resistance to electrical and heat stimuli. We attached the stimulating electrode (of electrical stimuli) on one of the participant's ventral forearms (counterbalanced across participants). The participant then rested for 2 min.

Assessing the current level that elicited a pain rating of 4 to electrical stimuli. To lessen the participant's pain experience during the procedure, we aimed for pain intensity that elicited a pain rating of 4 or "Pain-4." To personalise this Pain-4 Current Level for each participant, we applied an electrical stimulus, starting at a current level of 1 mA. The current level was then increased or decreased by 0.5 mA (with about 15-s break between each trial) until the participant gave a pain rating of 4. The exception was that, if the participant gave an initial pain rating below 3 or above 5, the current level was adjusted by 1 mA. It was to reduce the

number of delivering electrical stimuli to minimise skin sensitivity. The maximum current level was 10 mA. The finalised Pain-4 Current Level was used as the intensity for subsequent administration of electrical stimuli.

Assessing the temperature that elicited a pain rating of 4 to heat stimuli. To determine the temperature that elicited a pain rating of 4, the Pain-4 Temperature, we applied a heat stimulus for 7 s, starting at a temperature of 39 °C. The temperature was then increased or decreased by 1 °C until the participant gave a pain rating of 4. To minimise sensitisation at the actual conditioning site, we applied heat stimuli at skin areas 3-cm distant from the conditioning site and separated all trials by at least 30 s (Pedersen & Kehlet, 1998a, 1998b). Similarly, to reduce the number of applying heat stimuli, if the participant gave an initial pain rating below 3 or above 5, the temperature was increased or decreased by 2 °C. The maximum temperature was 43 °C. The finalised Pain-4 Temperature was used as the intensity for subsequent heat conditioning. Finally, the participant rested for 2 min.

Obtaining pain ratings during the CPM testing. Figure 2 (the next page) shows the time course, which took 10 min 30 s. Since the timer started, we applied the pre-conditioning electrical stimulus at 30 s. At 1 min, we started 5-min heat conditioning and asked for pain rating every minute (ended at 6 min). We applied the post-conditioning electrical stimulus at 5 min 30 s. Following that, we applied five more electrical stimuli, delivered 1 min apart. After obtaining the last pain rating, we removed all devices from the participant. The participant completed the exit survey (Appendix J) before leaving. The entire procedure took about 2 hr within one session.

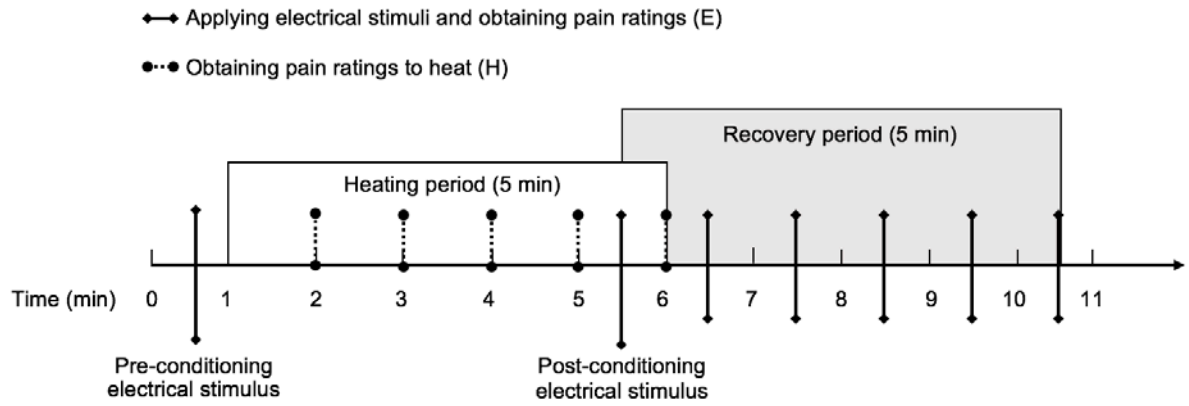

Figure 2. The time course of obtaining pain ratings during the conditioned pain modulation testing, which took 10 min 30 s. Overall, there were seven pain ratings to electrical stimuli (E) and five pain ratings to heat (H). The heating lasted for 5 min (from 1 to 6 min). The pre- and post-conditioning electrical stimuli were administered at 30 s and 5 min 30 s, respectively. Following that, five more electrical stimuli were delivered with a 1-min break during the recovery period.

Data Analysis

G*Power (Faul, Erdfelder, Buchner, & Lang, 2009) was used to calculate the sample size needed to yield a large effect size (f = .40; Cohen, 1988) with an alpha level of .05 and a power of .80. It suggested a sample size of 28 participants, which was unmet. Data were analysed using Statistical Package for Social Scientists (SPSS, standard version 25, 2017).

Testing assumptions of mixed model analysis of variance (ANOVA) and presenting effect sizes. Normality was tested by Shapiro-Wilk test, histogram, and boxplot. Homogeneity of variance within-group and between-groups were tested by F_{max} and Levene's tests, respectively. Sphericity was tested by Mauchly's test. Homogeneity of covariance matrices was tested by Box's *M* test. The partial eta squared (η_p^2) and Cohen's *d* were used as an index of effect size, where appropriate. By Cohen's (1988) convention, η_p^2 have a cut-off of .01

(*small*), .06 (*medium*), and .14 (*large*); Cohen's *d* has a cut-off of 0.20 (*small*), 0.50 (*medium*), and 0.80 (*large*). Where appropriate, 95% confidence intervals (CIs) were reported.

Assessing changes in pain ratings to electrical stimuli (E) across time between groups. A 2 × 7 mixed model ANOVA was used to assess changes in seven pain ratings to electrical stimuli across Time (E1, E2, E3, E4, E5, E6, E7) between Group (IBS, control).

Assessing changes in pain ratings to heat (H) during heating between groups. A 2 × 5 mixed model ANOVA was used to assess changes in five pain ratings to heat during Heating (H1, H2, H3, H4, H5) between Group (IBS, control).

Correlating the PCS scores with pain reports. Correlations between the PCS scores and pain ratings were run, separately for each group (N = 12), to examine the impact of pain anxiety on pain reports.

Results

Table 1 summarises participants' characteristics in each group (IBS, n = 12; control, n =12). The results of independent-sample *t* tests indicated no group differences in any of these variables (ps > .05).

Table 1

Participants' Characteristics in the IBS Group (n = 12) and the Control Group (n = 12)

Variable	IBS	Control	*p*	Cohen's *d*
Age	33.25 ± 14.99	27.42 ± 14.19	.338	0.40
Pain-4 Current Level	4.00 ± 2.44	3.21 ± 1.96	.390	0.36
Pain-4 Temperature	42.25 ± 1.48	41.17 ± 1.19	.062	0.80
PCS score	2.83 ± 3.06	3.53 ± 3.19	.592	0.22

Note. Results are presented as means ± standard deviations. IBS = irritable bowel syndrome; *p* = *p*-values of independent-sample *t* tests which had the criterion of statistical significance of .05; Cohen's *d* = the measure of effect size, with a cut-off of 0.20 (*small*), 0.50 (*medium*), and 0.80 (*large*) according to Cohen (1988); Pain-4 Current Level = the current level (mA) that elicited a pain rating of 4; Pain-4 Temperature = the temperature (°C) that elicited a pain rating of 4; PCS = Pain Catastrophizing Scale.

Changes in Pain Ratings to Electrical Stimuli (E) Across Time Between Groups

To examine the function of pain inhibition, a mixed model ANOVA was used to assess changes in seven pain ratings to electrical stimuli across Time (E1, E2, E3, E4, E5, E6, E7) between Group (IBS, control). It was hypothesised that the IBS group would have less pain inhibition than the controls.

Shapiro-Wilk test statistics indicated that the pain ratings of the IBS group at E1 was non-normal, $W(12) = 0.80$, $p = .009$; and the pain ratings of the control group were non-normal at E4 ($W(12) = 0.80$, $p = .010$), E5 ($W(12) = 0.76$, $p = .003$), E6 ($W(12) = 0.79$, $p = .007$), and E7 ($W(12) = 0.85$, $p = .037$). Visual inspection to relevant histograms and boxplots indicated positive skewness for every case. Both Skewness (z_S) and Kurtosis (z_K) statistics suggested mild violation to normality (*z*-values between ±1.96 and ±2.58). Thus, ANOVA was still robust. Mauchly's test indicated that sphericity was violated, $\chi^2(20) = 51.92$, $p < .001$, therefore degrees of freedom were corrected using Greenhouse-Geisser epsilon ($\varepsilon = .53$). The remaining assumptions were supported: F_{max} was 5.06, together with Levene's test statistics, demonstrating homogeneity of variance within-group and between-groups, respectively; Box's *M* test ($p = .461$) supported homogeneity of covariance matrices.

Figure 3 presents the results visually with error bars indicating 95% CIs. There was no significant main effect of Time, $F(3.17, 69.77) = 1.61$, $p = .192$, $\eta_p^2 = .07$, suggesting that seven pain ratings to electrical stimuli were similar across time irrespective of groups. There was no significant main effect of Group, $F(1, 22) = 0.14$, $p = .717$, $\eta_p^2 = .01$, suggesting that pain ratings to electrical stimuli were comparable between the IBS group ($M = 4.09$, 95% CI [3.40, 4.78], $SD = 1.63$) and the control group ($M = 3.92$ [3.23, 4.61], $SD = 1.63$). There was no Group × Time interaction, $F(3.17, 69.77) = 0.49$, $p = .699$, $\eta_p^2 = .02$. The results do not support the first hypothesis.

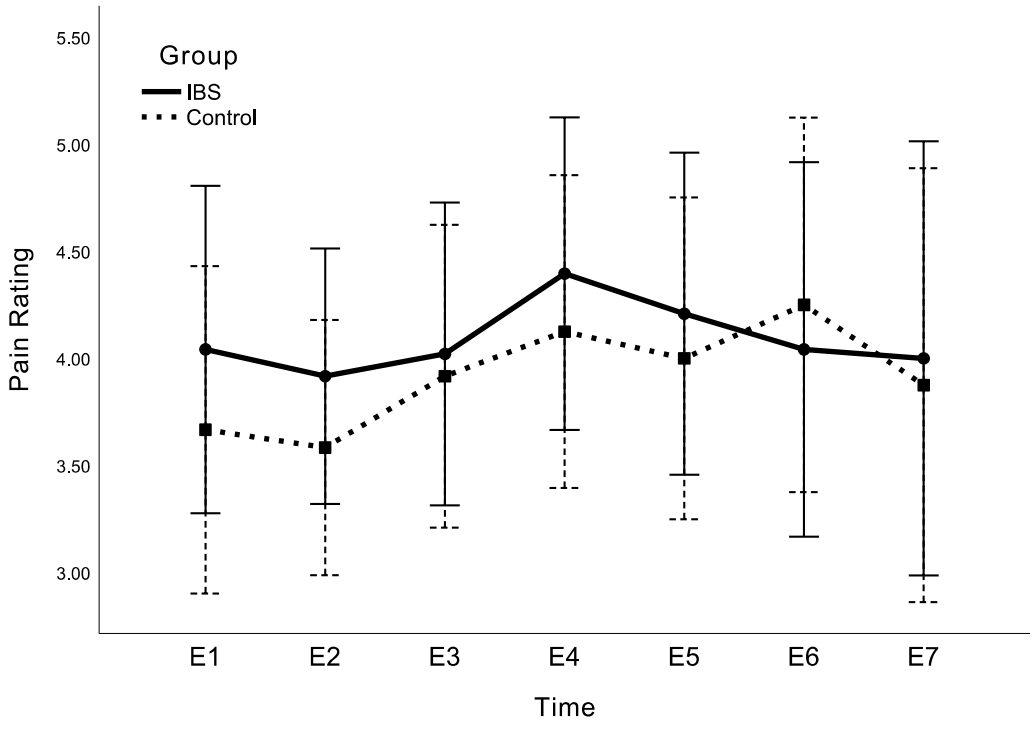

Figure 3. Group differences in changes of seven pain ratings to electrical stimuli (E) across Time. The irritable bowel syndrome (IBS) group and the control group had similar pain ratings to electrical stimuli across time (non-significant main effects of Group and Time). No Group × Time interaction. Error bars represent 95% confidence intervals.

Changes in Pain Ratings to Heat (H) During Heating Between Groups

To examine the speed of pain facilitation, a mixed model ANOVA was used to assess changes in five pain ratings to heat during Heating (H1, H2, H3, H4, H5) between Group (IBS, control). It was hypothesised that the IBS group would have more pain facilitation than the control group.

Shapiro-Wilk test statistics (*p*s > .05) and histograms indicated that all pain ratings to heat were normally distributed for both groups, boxplots suggested outliers though. F_{max} was 2.20, together with Levene's test statistics, demonstrating homogeneity of variance within-group and between-groups, respectively. Box's *M* test (*p* = .530) supported homogeneity of covariance matrices. However, Mauchly's test indicated that sphericity was violated, $\chi^2(9) = 59.28$, *p* < .001; thus, degrees of freedom were corrected using Greenhouse-Geisser epsilon ($\varepsilon = .41$).

Figure 4 (the next page) presents the results visually with error bars indicating 95% CIs. There was a significant main effect of Heating, $F(1.66, 36.45) = 11.59$, $p < .001$, $\eta_p^2 = .35$, suggesting that that pain ratings to heat increased in both groups. There was a significant main effect of Group, $F(1, 22) = 4.42$, $p = .047$, $\eta_p^2 = .17$. Pairwise comparison further revealed that the IBS group (*M* = 5.24 [4.30, 6.18], *SD* = 2.22) reported higher pain ratings to heat than the control group (*M* = 3.90 [2.96, 4.84], *SD* = 2.22). There was no Heating × Group interaction, $F(1.66, 36.45) = 1.31$, $p = .279$, $\eta_p^2 = .06$. The results support the second hypothesis.

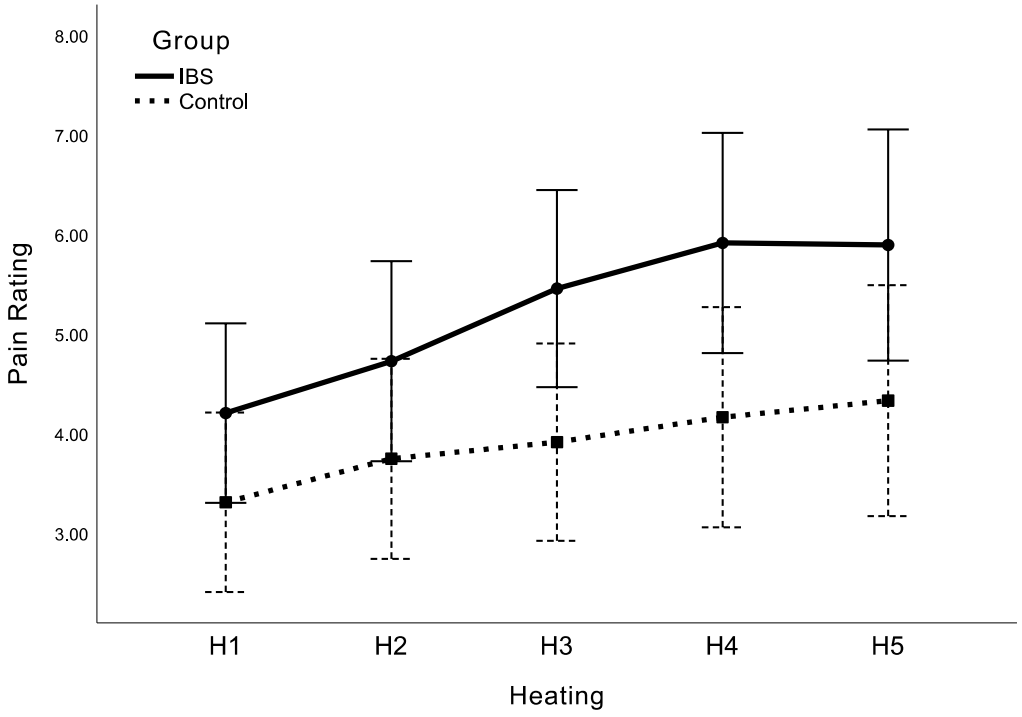

Figure 4. Group differences in changes of five pain ratings to heat (H) during Heating. Pain ratings increased during conditioning (significant main effect of Heating). The irritable bowel syndrome (IBS) group reported higher pain ratings than the controls (significant main effect of Group). No Heating × Group interaction. Error bars represent 95% confidence intervals.

Spearman's Rho (r_s) of Selected Variables

In addition to non-normal data of pain ratings to electrical stimuli (described above), Shapiro-Wilk test statistics indicated that the Pain-4 Current Levels of both the IBS group ($W(12) = 0.86$, $p = .045$) and the control group ($W(12) = 0.62$, $p < .001$) were not normally distributed; and the PCS score of the IBS group was non-normal ($W(12) = 0.83$, $p = .023$). Hence, Spearman's rho (r_s) was performed separately for each group. Tables 2 (the next page) summarises the correlations around seven pain ratings to electrical stimuli (E) of the IBS group ($N = 12$) and the control group ($N = 12$). Correlations around five pain ratings to heat were unrelated to the Pain-4 Current Level nor the Pain-4 Temperature (ps > .05), so the table was

omitted. The PCS scores of both groups were unrelated to the Pain-4 Current Level nor the Pain-4 Temperature, nor any pain ratings to electrical or heat stimuli (*p*s > .05).

Table 2

Spearman's Rho (r_s) Around Seven Pain Ratings to Electrical Stimuli (E) Across Time for the IBS Group (N = 12) and the Control Group (N = 12)

Variable	E1	E2	E3	E4	E5	E6	E7
IBS							
Pain-4 Current Level	-62*	-.09	-.42	-.31	-.31	-.54	-.05
Pain-4 Temperature	-.63*	.09	-.44	-.28	-.18	-.40	-.02
Control							
Pain-4 Current Level	-.19	-.04	-.43	-.33	-.02	-.17	.02
Pain-4 Temperature	-.37	-.59*	-.79**	-.66*	-.34	-.80**	-.54

Note. E1 = pain rating to the first electrical stimulus (pre-conditioning); E2 = the second (post-conditioning); E3 to E7 = the third to the seventh (during the recovery period); IBS = irritable bowel syndrome; Pain-4 Current Level = the current level (mA) that elicited a pain rating of 4; Pain-4 Temperature = the temperature (°C) that elicited a pain rating of 4.

*$p < .05$. **$p < .01$.

Discussion

The available evidence suggests that people with IBS experience chronic abdominal pain and have imbalanced GM composition and dysfunctional pain modulation. Therefore, this study aimed to examine the link between extreme BSS scores (reflecting imbalanced GM composition) and dysfunction pain modulation in women with versus without symptoms of IBS. Pain modulation (including inhibition and facilitation) was investigated using the CPM paradigm. It

was hypothesised that women in the IBS group would have less pain inhibition and more pain facilitation than the controls. The main findings were (a) both groups had no pain inhibition to electrical stimuli after heat conditioning, which does not support the first hypothesis; and (b) both groups exhibited pain facilitation during heating, but the heat pain accumulated faster in the IBS group than in the control group, which supports the second hypothesis. These results must be interpreted cautiously because of statistical and methodological issues.

Pain Inhibition: Changes in Pain Ratings to Electrical Stimuli (E) Across Time

The results do not support the first hypothesis that women in the IBS group would have less pain inhibition than the controls. Given the small effect size, these non-significant group differences are unlikely to be due to insufficient statistical power. Nevertheless, lack of pain inhibition in the IBS group after heating replicates previous findings, especially those, similar to the present study, which applied electrical test stimuli (Piche et al., 2010; Piche et al., 2011; Piche et al., 2013). Unlike these three studies, the control participants in the present study had no pain inhibition as well, which raises doubts about the validity of dysfunctional pain inhibition of the IBS group.

The severity of abdominal pain in the IBS participants may explain these non-significant group differences in pain inhibition. In most of the studies that found significant group differences, participants with IBS were recruited from the clinics (e.g., Piche et al., 2010; Piche et al., 2011; Piche et al., 2013). In two studies that found pain inhibition but no group differences, IBS participants were recruited from the community rather than the clinical centres (Jarrett et al., 2016; Jarrett et al., 2014). The IBS participants from the community may have less severe symptoms than those from the clinics, suggesting a link between increased symptom severity and decreased pain inhibition. In another study, Bouhassira et al. (2013) divided women

with IBS into two groups, as the IBS-*insensitive* group and the IBS-*sensitive* group. Their IBS-sensitive group included women with more severe IBS symptoms and greater pain sensitivity. Bouhassira et al. found that the IBS-sensitive group exhibited less pain inhibition than either the IBS-insensitive group or the control group (which had no differences between them). The authors of these three studies (Bouhassira et al., 2013; Jarrett et al., 2016; Jarrett et al., 2014) attributed the non-significant group differences to the severity of IBS symptoms. In line with the results from meta-analyses, more severe IBS symptoms linked to worse pain inhibition (Albusoda et al., 2018; Marcuzzi et al., 2019). Thus, this variable (severity) mattered.

In the present study, only five women in the IBS group met full diagnostic criteria for IBS and reported abdominal pain during the stool diary recording. Furthermore, as shown in Table 1 (p. 23), stimuli intensity to produce pain ratings of 4 (Pain-4 Current Levels and Pain-4 Temperatures) and PCS scores were similar in both groups. These results are not supported by previous studies that found significant group differences in pain inhibition (Piche et al., 2010; Piche et al., 2011; Piche et al., 2013). In their studies, the IBS group had pain hypersensitivity and higher PCS scores when compared with the controls. In other words, their results suggested that the IBS group in the present study should have tolerated lower current level and temperature and reported higher PCS scores than the controls; however, it was not the case. Hence, women in the IBS group might not have severe IBS symptoms. Given the link between more severe IBS symptoms and more severe dysbiosis (Liu et al., 2017; Pozuelo et al., 2015; Tap et al., 2017), the IBS participants and the controls in the present study may have had similar GM compositions. This similarity then potentially explains the non-significant group differences in pain inhibition. Future studies could include IBS severity scales (e.g., Francis, Morris, Whorwell, 1997) in addition to measures of GM composition for participants diagnosed with IBS.

After taking severity into account, present non-significant findings do not exclude a link between GM composition and pain inhibition, which warrants future examination. Nevertheless, the absence of pain inhibition in either group suggests a failure to address the hypothesis adequately in the current study. Possible reasons for this absence of pain inhibition are discussed below.

Preliminary pain sensitivity tests may have inhibited pain intensity to the pre-conditioning electrical stimulus. Before the actual CPM testing, preliminary pain sensitivity tests were used to obtain the Pain-4 Current Level and Pain-4 Temperature (refer to Procedure). The pain caused by these preliminary tests may have inhibited the pain caused by the pre-conditioning electrical stimulus. As a result, the rating of pre-conditioning electrical pain may have been lower than a pain rating of 4 already, which is supported by the descriptive statistics (Figure 3, p. 25). It then reduced differences (if any) between the ratings of the pre- and post-conditioning electrical pain. In other words, any pain inhibitory effect was masked.

According to the results of correlations (Table 2, p. 28), in the IBS group, the higher the Pain-4 Current Level and the Pain-4 Temperature, the lower the pain to the pre-conditioning electrical stimulus (E1). These results suggest that preliminary pain sensitivity tests elicited pain inhibition in the IBS group. However, this was not the case in the control group, where both the Pain-4 Current Level and the Pain-4 Temperature were unrelated to E1. As shown in Table 1 (p. 23), on average, the IBS participants had a Pain-4 Temperature that was 1-°C higher than the controls. Although these differences were not statistically significant, the effect size was large. Higher temperature means more preliminary heat tests because the temperature was adjusted in 1-°C steps. Hence, seemingly higher temperature (more tests, longer exposure to pain) of the IBS group may explain why preliminary heat tests were likely to inhibit E1 for the IBS group but

not for the controls. However, given the similar Pain-4 Current Levels between groups (and small effect size), the number of preliminary electrical tests was comparable in both groups. Thus, it is hard to explain why preliminary electrical tests were likely to inhibit E1 for the IBS groups but not for the controls. It is noteworthy that, for the control participants, although the Pain-4 Temperature was unrelated to E1, it had negative links to the rating to the post-conditioning electrical pain (E2) and the ratings of electrical pain during the recovery period (E3, E4, and E6). In other words, the higher the Pain-4 Temperature, the lower the ratings to electrical pain after heating. Therefore, it is tempting to consider these links as a sign of better function of pain inhibition in the control group. However, this speculation requires further investigation as the present study had a small sample size, and the data contained outliers.

In brief, the preliminary pain sensitivity tests may have inhibited the pain to the pre-conditioning electrical stimulus. Lower pre-conditioning electrical pain to begin with reduced the chance to observe differences between the pre- and post-conditioning electrical pain, which masked any pain inhibitory effect. This explanation has a few implications. First, the preliminary electrical tests were given separately with about 15-s break; the preliminary heat tests were applied for 7 s with at least 30-s break between each trial. If they elicited pain inhibition, they could be applied as conditioning stimuli in future CPM studies. In doing so, it would be easier for future studies to recruit participants because these preliminary tests were less painful than the 5-min heating applied in the present study (according to participants' feedback). Second, instead of 2-min rest after preliminary tests in the present study, a longer break is required in future studies. Previous studies, which found pain inhibition, applied a 5-min delay after preliminary tests (Jarrett et al., 2016; Jarrett et al., 2014). A safer way would be to arrange the preliminary tests and the main CPM procedure on different days, as King et al. (2009) did.

Mildly painful electrical test stimuli may have facilitated pain intensity to the post-conditioning electrical stimulus. In the CPM paradigm, the pain caused by the conditioning stimulus (CS) is supposed to inhibit the pain caused by the test stimulus (TS). However, there are individual differences in CPM responses. In healthy people, the strength and direction of CPM effect after conditioning depends on stimuli type, stimuli intensity, and body sites being applied for both the CS and the TS (Granot et al., 2008; Nahman-Averbuch et al., 2013; Pud, Granovsky, & Yarnitsky, 2009). Healthy people may exhibit no pain inhibition or even show pain facilitation *to the post-conditioning TS* (i.e., opposite direction; Potvin & Marchand, 2016). Note that this pain facilitation refers to increased pain intensity to the TS after conditioning, not as in the accumulation of pain to the CS during prolonged conditioning.

In a recent animal study, more intense TS tended to elicit the supposed pain inhibition, whereas less intense TS tended to elicit the opposite pain facilitation (Tansley et al., 2019). In the present study, a TS with low-to-moderate intensity (pain rating of 4) was used. It follows that the present study design may have triggered pain facilitatory influences on the TS after conditioning, which is supported by the descriptive statistics (Figure 3, p. 25). On average, the ratings of electrical pain during the recovery period increased slightly, which were not statistically significant (though the effect size was medium). Because this analysis was statistically underpowered, an association between the intensity of TS and the direction of CPM responses may have been missed.

In short, the mildly painful electrical TS used in present CPM testing may have facilitated the pain to the post-conditioning electrical stimulus so that any pain inhibitory effect was concealed. There are important implications for future studies. The CPM effect has value in predicting both chronic pain vulnerability and treatment responses (Lewis, Rice, & McNair,

2012; Niesters et al., 2014). However, in a recent meta-analysis, the CPM response is not currently a valid biomarker for chronic pain because of a large variation in the CPM testing design (Fernandes, Pidal-Miranda, Samartin-Veiga, & Carrillo-De-La-Peña, 2019). Misidentification can result in huge repercussions in clinical practice. For example, one may mistakenly conclude that the control participants were vulnerable to chronic pain because they had "dysfunctional" pain inhibition. This conclusion is misleading, given that the study may not have been appropriately designed to examine pain inhibition. Failing to identify people truly vulnerable to chronic pain is even more serious.

Other factors may also be crucial to consider in the CPM test paradigm. For example, in one study (Piche et al., 2010), the average baseline pain intensity to the TS was around 3.5 (out of 10), which was comparable to that in the present study. Nevertheless, Piche et al. (2010) still found a significant pain inhibitory effect of the CS on the TS. In the present study, the TS was applied to the forearm, whereas Piche et al. applied the TS on the leg. Different body sites reflect different spinal segments, where varying pain sensitivity has been observed (Zhou et al., 2010). Thus, it is imperative to have more studies, "from head to tail," examining the standardised CPM testing protocol.

Distress or anxiety about pain may have led to a lower-than-usual Pain-4 Current Level. Magnifying pain intensity is likely when people face stress (Sullivan et al., 2001). This phenomenon is in line with stress-induced hyperalgesia, an increased pain response after stress exposure (Martenson, Cetas, & Heinricher, 2009). As such, the participant, who felt anxious about pain, may have magnified pain intensity. So, she might give a pain rating of 4 to the current level normally eliciting her pain rating of 3, resulting in a lower-than-usual Pain-4 Current Level. If anxiety level decreased in the middle of the study (less magnification), the pre-

conditioning electrical pain might also decrease. Similarly, it reduced the chance to detect any pain inhibitory effect. On the other hand, if anxiety level increased during the study (more magnification), elevated pain rating to the post-conditioning electrical stimulus could also mask pain inhibitory effect.

In the present study, the PCS was used to measure participants' intensity of physical and emotional distress experienced during pain. If the above speculation is true, one could expect the Pain-4 Current Level to decrease as the PCS score increased (i.e., more distress, more magnification, lower Pain-4 Current Level). Similarly, the post-conditioning electrical pain would be expected to increase as the PCS score increased. There were no such links, suggesting that distress or anxiety about pain was unlikely to confound the results of pain inhibition. However, distress cannot be ruled out entirely. First, correlations may have been influenced by small sample size and outliers. Second, the PCS score obtained before the CPM testing did not represent the distress or anxiety levels during the testing. One way to handle this issue in future studies would be to measure distress together with pain intensity during the study, as other researchers did (e.g., Knudsen & Drummond, 2009; Piche et al., 2011; Piche et al., 2013).

Summary of pain inhibition. Results indicated no group differences in pain inhibition, which do not support the first hypothesis. It may be because the IBS participants did not have severe IBS symptoms, leading to fewer group differences in GM composition. Thus, these non-significant group differences do not exclude a link between GM composition and pain inhibition, which warrants future examination. Results also indicated that both groups exhibited no pain inhibition at all, suggesting potential confounds due to the study design. Possible explanations are (a) preliminary pain sensitivity tests may have inhibited the pain to the pre-conditioning electrical stimulus already, (b) mildly painful electrical test stimuli may have facilitated the pain

to the post-conditioning electrical stimulus, and (c) distress or anxiety about pain during the CPM testing may have led to pain magnification. Implications are to call for future studies to extend the delay between the preliminary and the actual testing procedure, to develop a standardised CPM testing protocol, and to measure distress or anxiety levels during the study.

Pain Facilitation: Changes in Pain Ratings to Heat (H) During Heating

The results support the second hypothesis that women in the IBS group would have greater pain facilitation during conditioning than the controls. Both groups had the same trend of pain facilitation (as the accumulation of heat pain), but the heat pain accumulated faster in the IBS group than in the control group. These results replicate a previous finding; the difference is that the present study found the accumulation of heat pain, whereas the accumulation of cold pain was investigated previously (Wilder-Smith & Robert-Yap, 2007). However, these results must be interpreted cautiously given the small sample size. Small sample size suggests that these significant group differences may have been due to chance and that the effect size may have been overestimated. Thus, it is important to replicate these findings in a large sample. Moreover, the following confounds that may have inflated the group differences need to be considered.

Two confounds heightening pain facilitation in the IBS group. First, on average, the IBS participants had a Pain-4 Temperature which was 1-°C higher than the controls, although these differences were not statistically significant. Nevertheless, the higher the starting temperature, the faster that heat pain accumulates (Weissman-Fogel, Dror, & Defrin, 2015). Thus, a relatively high temperature applied during heating may have augmented pain facilitation in the IBS group. It may be preferable to use a standardised rather than a personalised temperature for the heat stimulus in future studies. Second, the speed of pain facilitation may have been influenced by stress-induced hyperalgesia, an increased pain response after stress

exposure (Martenson et al., 2009; Sullivan et al., 2001). That is, the IBS participants may have magnified the heat pain during heating. However, because there was no link between the PCS scores and any heat pain ratings in the IBS group, the stress-induced hyperalgesia was unlikely to confound the group differences in pain facilitation.

Two confounds suppressing pain facilitation in the control group. First, instead of stress-induced hyperalgesia, people may show stress-induced analgesia, a reduced pain response after stress exposure (Yilmaz et al., 2010). That is, the control participants may have reported lower heat pain because of stress during heating. Because the PCS scores were unrelated to any ratings of heat pain in the control group, the stress-induced analgesia was unlikely a confound. Second, the unpleasantness of the heat stimulus mattered in the present study. Two IBS participants and two controls reported that "heating was warm and comfortable" after testing, probably because the data collection was in the winter. The impact of pleasantness cannot be explored because the present study did not measure the unpleasantness level as other researchers did (e.g., Knudsen, Finch, & Drummond, 2011; Piche et al., 2010). Thus, future studies should always measure the unpleasantness of painful stimuli, together with pain intensity.

Summary of pain facilitation. Results revealed that the IBS group had greater pain facilitation than the controls, which support the second hypothesis and warrant future examination. Although the results provide tentative support for a link between GM composition and pain facilitation, these significant group differences may have been due to chance. It is crucial to replicate these findings in a large sample. Additionally, future studies could control confounds that potentially inflated the group differences by (a) standardising the temperature of the heat stimulus, (b) measuring distress or anxiety levels during the testing, and (c) measuring the unpleasantness of painful stimuli together with pain intensity.

Methodological Consideration

This study has limitations, which are listed below together with possible solutions. First and foremost, it is unsure the extent to which the link between diarrhoea or constipation and faster accumulation of heat pain reflects the link between GM composition and pain modulation. Diarrhoea or constipation may not represent GM composition under certain circumstances. For instance, as in dehydration-induced constipation or magnesium-induced diarrhoea (Donowitz, 1991). A related issue is a doubt about the validity of group allocation that represented different GM compositions. For example, the IBS participants might have healthy GM composition but reported constipation (because of dehydration) or diarrhoea (because of high magnesium intake). It is also possible that the control participants had constipation but reported normal stool patterns. Constipation does not necessarily mean harder stools; it also implies infrequent or incomplete defecation (Lacy et al., 2016). Furthermore, this self-reported stool consistency, measured on the BSS, causes concern. Despite high overall accuracy and reliability of the BSS, it is hard to distinguish Type 2 (constipation) from Type 3 (normal) or to differentiate Type 5 (normal) from Type 6 (diarrhoea; Blake et al., 2016). In brief, this study did not use an accurate measure of GM composition, which undermined the validity of the results. Advanced stool analysis (e.g., Navas-Molina et al., 2013) is necessary for studying GM composition, which future studies could adopt.

Second, only women participated in this study, female sex hormones not only affect GM composition and stool consistency (García-Gómez, González-Pedrajo, & Camacho-Arroyo, 2013; Menon et al., 2013) but also influence pain perception (Meleine & Matricon, 2014). Specifically, the pain becomes worse during menstruation phase (characterised by low estrogen and progesterone levels). Two control participants were menstruating on the day of testing,

which means that they may have been hypersensitive to pain on that day. Future studies could conduct the testing after the participant's menstruation ends to address this issue.

Third, this study relied on a self-reported measure of pain intensity by a verbal pain rating scale. Although participants were blind to the hypotheses, individual differences in conceptualising pain were unavoidable. This issue remains unsolved in pain research. Future studies are needed to identify how to measure pain intensity objectively in humans.

Lastly, this study had a small sample size, which limited data exploration. The small sample size did not allow to compare differences in pain modulation between healthy women with diarrhoea or constipation and healthy women with normal stool consistency. It also did not allow to compare differences in pain modulation among IBS subtypes. Additionally, this study examined women only, which limits the generalisability of the findings. There may be more similarities than differences in IBS symptoms between men and women (Björkman, Jakobsson Ung, Ringström, Törnblom, & Simrén, 2015). Future studies could examine both men and women in a large sample.

Conclusion and Implications

The cause of chronic abdominal pain in IBS remains unclear. This study aimed to bridge a gap between GM composition (a recently proposed cause) with pain modulation (an established cause). GM composition was inferred by stool consistency; pain modulation was tested using the CPM paradigm. First, results indicated that women with looser or harder stool consistency (reflecting imbalanced GM composition) and women with normal stool consistency (healthy GM composition) had no pain inhibition to electrical stimuli after heat conditioning. Lack of group differences may be because women with diarrhoea or constipation did not have severe IBS symptoms. Lack of pain inhibition in either group may be due to confounds in this

study that either decreased the pre-conditioning pain or increased the post-conditioning pain. These confounds then masked any pain inhibitory effect. Second, results indicated that women with diarrhoea or constipation exhibited a faster accumulation of heat pain during heating than women with normal stools. These findings suggest a link between imbalanced GM composition and heightened pain facilitation. Future pain studies in healthy people may consider excluding participants who experience diarrhoea or constipation. Although this study provides tentative support and warrants future examination, results must be interpreted cautiously because of statistical and methodological issues. Major implications for future studies are (a) to develop a standardised CPM testing protocol, (b) to measure distress or anxiety levels during the study, (c) to measure the unpleasantness of painful stimuli together with pain intensity, (d) to use an accurate measure of GM composition, and (e) to replicate the findings in large sample.

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