FUNCTIONAL MAGNETIC RESONANCE IMAGING OF THE SPINAL CORD/BRAINSTEM AND BRAIN IN WOMEN WITH PROVOKED VESTIBULODYNIA

by

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Abstract

Vulvodynia (i.e., idiopathic chronic pain affecting the vulva) is a common but poorly understood pain condition, affecting 16% of women in the general population and leading to negative impacts in many life domains, including psychosocial function and reproductive potential (Pukall et al., 2016). An emerging body of research suggests that central factors (i.e., those involving the brain and spinal cord) may play a role in the etiology and/or maintenance of vulvodynia (Pukall et al., 2016).

Functional magnetic resonance imaging (fMRI) studies have indicated that women with provoked vestibulodynia (PVD)—a form of vulvodynia characterized by provoked pain in response to pressure applied to the vaginal entrance—exhibit increased neural activity in response to both genital and non-genital stimulation in cortical areas related to pain modulation (Hampson et al., 2013; Pukall et al., 2005; Sutton et al., 2015; Pazmany et al., 2017). However, despite the fundamental role of the brainstem and spinal cord in descending modulation of pain, no studies have attempted to examine activation patterns in the spinal cord of women with vulvodynia.

The aim of this study was to examine spinal cord and brain connectivity and activation in women with PVD during painful hand stimulation to evoke descending modulation, the process responsible for the descending control of pain involving brain, supraspinal, and spinal structures (Gebhart, 2004). We hypothesized that women with PVD would exhibit altered connectivity in regions of the brain and spinal cord, indicating diminished descending modulation, and would also have greater blood oxygen level dependent (BOLD) responses in regions previously associated with pain processing. The results of this study examining the spinal cord/brainstem revealed alterations in the connectivity and BOLD responses of regions related to pain

modulation. Investigation of the brain revealed similar regions of connectivity in women with PVD and control women; however, this study adds to a growing body of research that have found alterations in the fMRI response of these brain regions in women with PVD. Through furthering our knowledge of spinally-mediated pain mechanisms in PVD, this study provides a more comprehensive understanding of the role of central processing in women with PVD.

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ACC	anterior cingulate cortex
Amyg	amygdala
BOLD	blood oxygenation-level dependent
C6	the 6th cervical spinal cord segment
CC/B	cervical cord/brainstem
CNS	central nervous system
CPM	conditioned pain modulation
CSF	cerebrospinal fluid
DRt	dorsal reticular nucleus
EPI	echo-planar imaging
fMRI	functional magnetic resonance imaging
GLM	general linear model
GPPPD	genito-pelvic pain/penetration disorder
HASTE	half-fourier single-shot fast spin-echo
IBS	irritable bowel syndrome
INS	insular cortex
LC	locus coeruleus
MRI	magnetic resonance imaging
NGC	nucleus gigantocellularis
NRM	nucleus raphe magnus
NTS	nucleus tractus solitarius

PAG	periaqueductal gray matter
PBN	parabrachial nucleus
PCC	posterior cingulate cortex
PFC	prefrontal cortex
PPC	posterior parietal cortex
PVD	provoked vestibulodynia
QST	quantitative sensory testing
ROI	region of interest
RTS-1	Robotic Thermal Stimulator-1
RVM	rostral ventromedial medulla
SEM	structural equation modeling
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SC	spinal cord
TE	echo time
Thal	thalamus
TR	repetition time

Chapter 1

Introduction

Provoked vestibulodynia (PVD) is the most common subtype of vulvodynia—defined as idiopathic chronic vulvar pain (Bornstein et al., 2016)—that has been estimated to affect 16% of women in the general population (Harlow, Wise, & Stewart, 2001). PVD is characterized by pain evoked by pressure to the vulvar region. The pain of PVD can occur in response to sexual or nonsexual situations, and is often described as intense, stabbing, and burning (Bergeron, Binik, Khalifé, Pagidas, & Glazer, 2001). The pain of PVD can be present from the first time that vaginal penetration is attempted (primary PVD), or it may develop after a period of pain-free vaginal penetration activities (secondary PVD; Pukall, 2016). Women with PVD experience negative impacts in terms of their mental health (e.g., increases in anxiety and depressive symptoms), sexual function (e.g., reduced pleasure and intercourse frequency), and relationship adjustment (e.g., more conflict), and they report decreases in their reproductive potential and overall quality of life (Pukall et al., 2016). However, the negative impacts are not limited to just those with PVD; partners may also experience psychological distress (Jodoin et al., 2008) and sexual issues (Smith & Pukall, 2014). Indeed, healthy partnership dynamics are reduced in PVD-affected couples (Pukall et al., 2016).

Many pathophysiological factors have been implicated in the expression of PVD (Pukall et al., 2016), such that different etiological pathways may play a role in the initiation and maintenance of PVD in different patients. Vulvodynia has previously been found to be commonly comorbid with other chronic pain conditions, most notably fibromyalgia and irritable bowel syndrome (Arnold, Bachmann, Kelly, Rosen, & Rhoads, 2006). This high comorbidity has led to some speculation that perhaps these frequently comorbid, idiopathic pain disorders are

indicative of a global pain syndrome (White & Harth, 2001; Arnold et al., 2006). However, the majority of previous work examining the etiology of PVD have focused on abnormalities in the vulva (e.g., inflammation). A previous popular theory of the pathology of PVD was based on neurogenic inflammation, stating that the release of pro-inflammatory cytokines in response to some form of injury (e.g., repeated yeast infections) sensitizes nociceptors, leading to amplified sensitivity. Supporting this theory, early studies reported increased inflammatory cytokines in vulvar biopsy samples taken from women with vulvodynia when compared to those taken from pain-free participants (e.g., mast cells; Bornstein, Goldschmid, & Sabo, 2004); however, other studies found no such evidence (e.g., Halperin et al., 2005). Despite this controversy, controlled studies examining vulvar sensitivity in women with PVD reported a pattern of hypersensitivity to almost all stimulus modalities tested in affected women (see Pukall & Cahill, 2014 for a review), supporting the idea that changes in the vestibular tissue (e.g., hyperinnervation; Bornstein et al., 2004) may be implicated in the expression of PVD.

Quantitative Sensory Testing (QST) of Vulvar Regions in PVD

Numerous studies have examined the peripheral sensitivity of women with PVD using quantitative sensory testing (QST) methods (e.g., punctate tactile, pressure pain, and thermal), specifically focusing on the vulvar region. Findings from these studies demonstrate striking evidence of hypersensitivity in women with PVD, specifically allodynia (i.e., pain in response to a normally non-painful stimulus) and hyperalgesia (i.e., an increased response to a normally painful stimulus) to almost all stimulus modalities. Women with vulvodynia have been shown to experience vulvar allodynia to mechanical pain testing and exhibit lower thresholds to both heat and cold pain sensations as compared to control women (Bohm-Starke, Hilliges, Brodda-Jansen, Rylander, & Torebjörk, 2001; Giesecke et al., 2004). Pukall et al. reported similar results, in that

women with PVD had lower vulvar tactile and pain thresholds than control women (Pukall, Binik, Khalifé, Amsel, & Abbott, 2002); however, they also examined sensitivity to various forms of stimuli over the deltoid muscle in order to examine whether the sensory dysregulation extended beyond the vulvar region. They found that women with PVD also exhibited lower thresholds in this region and some other non-vulvar areas. A few studies have similarly begun to expand QST investigations into non-vulvar regions in order to examine the possibility of generalized sensitization in women with PVD.

QST of Non-Vulvar Regions in PVD

Studies have examined sensitization beyond the vulvar region via QST and have demonstrated hypersensitivity in women with PVD as compared to control women (e.g., Pukall, Baron, Amsel, Khalife, & Binik, 2006; Bohm-Starke et al., 2001; Giesecke et al., 2004; Granot, Friedman, Yarnitsky, & Zimmer, 2002). This pattern has also been supported by self-report measures, as it has been found that women with PVD, on average, report more bodily pain than non-affected women (Danielsson, Eisemann, Sjoberg, & Wikman, 2001; Pukall et al., 2006). This body of research suggests that central factors, involving the brain and spinal cord, may play a role in the expression of vulvodynia in general, and of PVD specifically, in addition to regionally localized factors (e.g., inflammatory processes). A number of non-vulvar locations, including the thumb, forearm, deltoid, and shin, have been shown to exhibit hypersensitivity in women with vulvodynia/PVD in response to pressure pain via QST or manual pressure (Pukall et al., 2002; Pukall et al., 2006; Giesecke et al., 2004; Granot et al., 2002). In addition, Pukall et al. (2006) examined pain intensity and unpleasantness ratings of women with and without PVD during a tender point exam (via manual palpation) of nine bilateral non-vulvar areas and found higher ratings in the PVD group as compared to the control group. These sensory abnormalities,

supported by increases in pain reports, in non-vulvar regions suggest a widespread hypersensitivity, possibly as a result of central sensitization (Pukall & Cahill, 2014). Peripheral sensitization in women with vulvodynia/PVD has also been assessed using QST-based studies investigating changes in thresholds in response to heat pain applied to the forearm (Granot et al., 2002) and receptive fields following capsaicin injections into the forearm and foot (Foster, Dworkin, & Wood, 2005). Granot et al. concluded that women with vulvodynia experienced systemic pain perception, as displayed by the reduction in their unpleasantness and pain thresholds to pain applied to the forearm. Participants with vulvodynia also exhibit an expanded receptive field to painful stimuli following capsaicin injections, an enhancement of pain sensitivity with intra-dermal capsaicin, and allodynia in response to a non-painful, cotton-swab spring apparatus (Foster et al., 2005). Both the hyperalgesia, observed as a reduction in pain thresholds, and allodynia, measured as a pain response to non-noxious stimuli, reported in these studies are defining characteristics of central sensitization (Pukall & Cahill, 2014). This process has been suggested to contribute to the clinical presentation of women with vulvodynia (Pukall et al., 2002). The possibility of modified central processes has resulted in a number of investigations into neural activation patterns in women with vulvodynia/PVD.

Conditioned Pain Modulation

Based on previous research, there is reasonable support for the alteration of central factors such as sensitization and the dysregulation of endogenous pain modulatory systems in women with PVD. Conditioned Pain Modulation (CPM) is a pain inhibitory system that has been shown to exhibit decreases in patients with numerous chronic pain conditions. CPM, when intact, leads to the inhibition of a pain response by the application of a painful stimulus at a distal body site ("pain inhibiting pain"). Studies of CPM test the integrity of central pain modulatory

mechanisms (Lautenbacher & Rollman, 1997), in particular, the supraspinal structures (e.g. brainstem), where much of the pain inhibitory mechanisms originate (Edwards, Ness, & Weigent, 2003). Patients with chronic pain conditions (e.g., IBS) typically exhibit an absent or significantly diminished CPM response (Bouhassira et al., 2013; Normand et al., 2011), such that they lack inhibition of pain, leading to amplification of pain intensity. However, the examination of CPM among women with PVD has not led to a similar pattern of results.

Johanesson et al. (2007) investigated the inhibition of pressure pain to the contralateral leg in conjunction with a cold pressor test (i.e., immersion of one's arm into an ice-cold water bath). Despite showing hypersensitivity to the pressure stimuli, women with PVD had *higher* pain thresholds during the cold-water task than their reported thresholds before the task, indicating intact CPM. Similar results were found using heat pain as the measured stimulus (Sutton, Pukall, & Chamberlain, 2012); it is possible that these studies relied on QST stimuli that may not have been intense enough to examine the pain inhibitory system initiated by a pain response. However, the reliability of the CPM mechanism is a controversial topic, as a body of research has reported in groups of healthy, pain-free participants, some seem to show evidence of "inefficient" CPM. Inefficient CPM is described as the alternate pain stimulus actually facilitating the target pain condition instead of inhibiting it (Sprenger, Bingel, & Büchel, 2011; Bogdanov, 2015; Piché, Arsenault, & Rainville, 2009; Cotoi, 2015). These mixed results create some hesitancy when interpreting CPM results, as one would hope to see consistently intact inhibition in healthy populations if "inefficient" CPM is evident of a dysregulation in pain processing. Although measurement of CPM can give an indication of central pain modulatory mechanisms, imaging protocols are a direct way to examine blood oxygen-level dependent (BOLD)—the measurement of blood oxygenation known to be related to neural activity and

oxygen demand—not only in the brain but also in the spinal cord in order to assess inhibitory and facilitatory systems that are mediated by supraspinal structures.

Neural Activation Patterns in Women with Vulvodynia/PVD

Studies of brain activation lend support to QST evidence that central processes may be involved in the peripheral sensitization observed in women with PVD (Hampson et al., 2013; Pukall et al., 2005). Functional and structural brain imaging methods are popular means of studying neural correlates of pain, but although such studies are common in the general chronic pain literature, only six studies have been published to date examining neural correlates of pain in women with vulvodynia/PVD. Of these six studies, the first investigated neural correlates of both vulvar pain and touch in women with PVD using functional magnetic resonance imaging (fMRI). Women with PVD were found to have augmented neural activity in brain areas such as the somatosensory, insular, and anterior cingulate regions in response to a vulvar pain stimulus (Pukall et al., 2005). These regions have previously been found to play a role in pain-related activation (Coghill, Sang, Maisog, & Iadarola, 1999; Hofbauer, Rainville, & Duncan, 2001; Casey, Lorenz, & Minoshima, 2003). In addition, non-painful vestibular pressure led to significantly higher activation levels in the insular, frontal, and somatosensory regions in women with PVD than pain-free control women (Pukall et al., 2005); similar evidence for increased perception of innocuous stimuli has been found in other chronic pain conditions (Gracely, Petzke, Wolf, & Clauw, 2002; Hofbauer, Olausson, & Bushnell, 2006; Drossman et al., 2003).

Most recently, a study of women diagnosed with Genito-Pelvic Pain/Penetration Disorder (GPPPD)—a diagnostic label which includes vulvodynia and its subtypes—used individually titrated moderate pressure pain stimuli on the vulvar vestibule while undergoing fMRI scanning (Pazmany et al., 2017). Because women with GPPPD report higher levels of catastrophizing,

hypervigilance to pain, and pain-related fear and anxiety, the researchers sought to measure the cognitive aspects of pain perception by focusing on regions of affective processing. The authors found that brain activation during a painful stimulus on the vestibule was increased for those with GPPPD as compared to nonaffected women, primarily in regions related to cognitive and affective pain processing, such as the parahippocampal gyrus, amygdala, and hippocampus. However, they did not find any significant differences between women with GPPPD and healthy controls during periods of pain anticipation as hypothesized.

Differences in neural activation have also been seen between women with primary and secondary PVD; women with primary PVD show evidence of heightened activation in the left MCC, right putamen, and left middle frontal gyrus—areas important for pain processing—as compared to those with secondary PVD (Sutton, Pukall, Wild, Johnsrude, & Chamberlain, 2015). The putamen, for instance, has been found to be related to chronic and acute pain processing and is not active when a painful stimulus is not present (Borsook, Sava, & Becerra, 2010; Coghill et al., 1999; Downar, Mikulis, & Davis, 2003; Oertel, Preibisch, Martin, Walter, Gamer, Deichmann, & Lötsch, 2012). These results contribute some evidence to theories that primary and secondary PVD do not share a common etiology, and that primary PVD could be more related to alterations in central processes (Goetsch, 1991; Granot, Friedman, Yarnitsky, Tamir, & Zimmer, 2004; Sutton et al., 2015).

As with QST studies of women with vulvodynia/PVD, the investigation of neural activation in response to pain has not been limited to stimuli applied to the vulvar region. Hampson et al. (2013) examined both women with vulvodynia, some of whom had PVD, and women with fibromyalgia (as a positive control group) in response to vulvar and thumb pain stimuli adapted for each participant to elicit the same pain rating. Although women with

vulvodynia did not differ in terms of activation from control women in response to vulvar pain, they exhibited more insular activation as well as higher activation in the dorsal midcingulate, posterior cingulate, and ventral posteriolateral nuclei of the thalamus in response to "slightly intense" pressure pain. These areas are all commonly activated during the experience of pain and sensory integration for pain processing (Casey, 1996; Derbyshire, 2007). Activations were found to be correlated with self-reported clinical pain assessed via the McGill Pain Questionnaire (Melzack, & Katz, 2007). Findings also showed that the vulvodynia and fibromyalgia groups demonstrated higher activation levels than control women in areas such as the secondary somatosensory cortex, anterior insula, and mid-insula in response to "painful" thumb pressure (Hampson et al., 2013). The heightened overlapping activity in the insula was interpreted as further evidence that vulvodynia and fibromyalgia share biological characteristics.

It has also been demonstrated that the resting brains of women with PVD—during a task-free fMRI protocol—show alterations in neural networks. Previous fMRI research has shown these networks are related to body sensation awareness and the generation of appropriate motor responses to sensory input from the periphery (sensorimotor network), homeostatic body monitoring via millisecond adjustments to the autonomic nervous system and behavioral responses in response to changes—real or anticipated—in the environment (salience network), and resting as well as self-referential thinking (default mode network) as compared to women with irritable bowel syndrome (IBS) and control women (Gupta et al., 2015; Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995; Borsook, Edwards, Elman, Becerra, & Levine, 2013; Molnar-Szakacs, & Uddin, 2013). Similar alterations to the aforementioned networks have been demonstrated in other chronic pain conditions (Gupta et al., 2014), some of which are commonly comorbid with PVD (e.g., IBS and fibromyalgia) (Nguyen, Veasley, & Smolenski, 2013; Reed et

al., 2012), suggesting a shared source of dysfunction. Indeed, Gupta et al. (2015) found some overlap between the resting state activation in regions related to these networks of women with PVD and IBS. They further demonstrated that women with PVD showed some substantial alterations in intrinsic connectivity in regions comprising these three networks as compared with healthy control women and women with IBS. These changes in the connectivity of three networks were found to be moderately correlated with clinical outcomes, such as intensity of vulvar pain and vaginal muscle tenderness. Often research of chronic pain conditions seeks to find common characteristics between conditions; however, these results suggest some disease specificity for central pathophysiology. When combining these results, patterns reveal neural evidence for augmented sensory processing, as well as possible evidence for the involvement of central neural pathways in pain processing and generalized hyperalgesia of patients with PVD.

Structural Changes in Women with Vulvodynia

In addition to the changes in activation patterns investigated in functional imaging, anatomical neuroimaging of women with PVD has also reported structural differences. Areas important to pain processing and stress responses, such as the parahippocampal gyrus/hippocampus and basal ganglia, have been found to have increased gray matter density in women with PVD; in addition, increases in gray matter density in many of these regions, including the parahippocampus, globus pallidus, and substantia nigra, were related to clinical symptoms such as a lowered pain threshold (Schweinhardt, Kuchinad, Pukall, & Bushnell, 2008). This increase is in direct juxtaposition with studies of gray matter density in other chronic pain populations such as fibromyalgia, chronic low back pain, and tension headaches, in which there is typically a decrease (Kuchinad et al., 2007; Apkarian et al., 2004; Schmidt-Wilcke et al., 2005). However, as explained by Schweinhardt et al., these conflicting results could be explained

by the inherent difference between a continuous, spontaneous condition such as fibromyalgia and a provoked condition like PVD. Another study indicated that gray matter density also differed between women with primary and secondary PVD; women with primary PVD showed significant decreases in gray matter throughout brain areas associated with pain processing as compared to women with secondary PVD (Sutton et al., 2015). These results suggest that perhaps there are differing pain processing characteristics between women with primary and secondary PVD, as significantly decreased gray matter in women with primary PVD is characteristic of chronic pain conditions, and this study confirmed previous results that found women with primary PVD have lower pain thresholds. The conclusions drawn from these studies provide evidence for the correlation between heightened sensitivity to stimulation in women with PVD and changes in neural activation patterns and morphological changes as assessed by imaging techniques.

Descending Modulation of Pain in Supraspinal Structures

It is now recognized that brainstem structures, through the use of descending facilitatory and inhibitory neurons, can increase or suppress pain and sensory information that is projected from the spinal cord to the brain (Bee & Dickenson, 2009). There are both "bottom-up" and "top-down" pathways that influence pain perception between the spinal cord and brain (Staud, 2013). For instance, the spinothalamic tract is one of several primary ascending pathways for transmitting pain information to the brainstem and thalamus. Descending pathways from the cortex modulate these ascending projections and therefore influence how we perceive pain (Staud, 2013; Stroman, 2011). Descending pain modulation is mediated by interconnected brainstem structures that project to the dorsal horn of the spinal cord (Cahill & Stroman, 2011; Staud, 2013). For instance, both the rostroventromedial medulla (RVM) and nucleus tractus

solitarius (NTS) are the origin of both descending facilitatory and inhibitory pathways (Millan, 2001).

The RVM, NTS, and parabrachial nucleus (PBN) are all interconnected loci of the brainstem that give information to, and receive information from, the spinal cord (Millan, 2001). Both the NTS and PBN are important for integrating sensory and autonomic information and the descending modulation of pain. Indeed, stimulation of the NTS has been shown to evoke antinociceptive effects (Millan, 2001). These brainstem regions are connected with the dorsal horn, and they play a role in the inhibition of pain; they can also elicit hyperalgesia when stimulated, as has been shown with the dorsal reticular nucleus (DRt) (Millan, 2001). The nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis (NGC) are subdivisions of the RVM that project to the dorsal horn. Cells found in the RVM are known to both facilitate (i.e. ON cells) or inhibit (i.e. OFF cells) pain (Staud, 2013). These descending mechanisms are modified by other supraspinal structures, including the PBN, NTS, and periaqueductal grey (PAG) (Millan, 2001). For these reasons, when investigating alterations of CNS pain processing, the examination of spinal cord/brainstem regions responsible for the descending modulation of pain is necessary, in addition to brain regions.

CC/B (Cervical Cord/Brainstem) Imaging Protocol

Despite the direction of the literature towards the examination of central pain processes, no studies have yet attempted to examine activation patterns in the spinal cord as well as the brain of women with PVD. This gap may be due to the complex nature of spinal cord imaging, even though an established protocol exists. In order to fully understand the alterations in regulation of pain, the proposed study aims to take the next step in investigating descending modulation in the spinal cord of women with and without PVD. Despite the complications

created by the spinal cord for fMRI, many studies have successfully adapted fMRI protocols using an fMRI data acquisition for brainstem and spinal cord imaging (Harita & Stroman, 2017; Bosma et al., 2016; Bosma & Stroman, 2014). The anatomical structure of the spinal cord presents many challenges to fMRI, such as bone and tissue field variance, movement of the spine itself, and movement caused by cardiac and respiratory cycles. In addition, the spinal cord is within the spinal canal of the spine and moves with the flow of cerebrospinal fluid (CSF) (Figley and Stroman, 2007). The proximity to the lungs and hearts also create artifacts due to movement related to respiration. In order to account for the challenges created by the spinal cord, a data analysis package has been developed by P. Stroman to remove noise caused by motion and map image data onto a normalized template (Bosma et al., 2014). This protocol has successfully been used in healthy participants under multiple pain manipulations (Khan & Stroman, 2015; Stroman et al., 2016) as well as in populations with spinal cord injuries and fibromyalgia (Stroman et al., 2016; Bosma et al., 2016). These studies have demonstrated effects of cognitive and emotional modulation of pain via descending pain modulation systems, effects of central sensitization, and effects of peripheral changes in noxious input (Bosma, Mojarad, Leung, Pukall, Staud, & Stroman, 2015; Bosma & Stroman, 2015; Cadotte et al., 2012; Dobek, Beynon, Bosma et al., 2014; Khan et al., 2015). In addition, recent results observing brain and CC/B activation in anticipation of a stimulus or absence of a stimulus using this protocol have demonstrated continuous descending modulation in spinal cord neurons (Stroman et al., 2016). It is our expectation that these processes will be modified in groups with chronic pain conditions such as PVD and that these alterations will be related to their emotional and cognitive state.

Current Study

Taken together, these results suggest that women with PVD may differ in their peripheral and central processing of pain and sensory stimuli, and a method now exists for directly investigating these differences. The current study examined alterations in BOLD responses believed to be the result of activation in the brain and spinal cord in response to a controlled heat stimulus. BOLD responses in relevant pain processing regions were measured using fMRI as well as subjective pain ratings. The aim of the current study was to evoke global descending modulation pain mechanisms and investigate the differences between women with PVD (including those with both primary and secondary PVD) and control women. The primary hypothesis is that heightened pain sensitivity in PVD will be related to altered physiological processes in the spinal cord and brainstem. We more specifically hypothesized that women with PVD would:

1. Exhibit altered connectivity in regions known to be related to pain processing in the brain and brainstem/spinal cord, indicating diminished descending modulation.

2. Have greater BOLD responses in response to the pain stimulation paradigm in regions known to be related to pain processing.

Examination of brainstem/spinal cord responses to painful stimulation has not yet been examined in women with PVD. Regions of interest investigated in this study were based on previous similar studies of chronic pain conditions such as fibromyalgia; for this reason, this study is exploratory in nature. The results from this study may provide new insights into human pain processing and how it is altered in chronic pain conditions, as well as contribute to the PVD literature.

Chapter 2

Method

Participants

Recruitment. Prospective participants were recruited through several means: (1) the Sexual Health Research Laboratory (SexLab) database, consisting of past participants who have consented to being contacted for participation in future research, (2) posted advertisements around the Kingston community, with a focus on the Queen's University campus and downtown area, (3) social media (e.g., Facebook) advertisements, (4) pamphlets placed in doctors' offices, and (5) emails to colleagues within the relevant fields in contact with the population (e.g. urologists, gynecologists). Potential participants who were interested underwent a telephone or online screening process to assess their eligibility. Figure 5 illustrates the study procedure and the number of participants who completed each phase.

Screening. Eligibility screening was done either over the phone or online using Qualtrics survey software. Thirty-two women between the ages of 18 and 48 years were recruited for this study, 16 women in each group (i.e., PVD, control). Demographic information for these participants can be found in Table 1. The groups were matched on age (+/- 5 years) to account for age-related changes in sensory processing (Lautenbacher, Kunz, Strate, Nielsen, & Arendt-Nielsen, 2005), and on current hormonal contraceptive use (yes or no) to account for any potential effects (e.g., on pain sensitivity) of exogenous hormones (Vincent & Tracey, 2008; Wilson & Tashani, 2016). Exclusion criteria for participation included: being under the age of 18 or over the age of 50; presence of magnetic resonance imaging (MRI) contraindications (e.g., metal implants); major brain or spinal cord injury; the use of medications that substantially affect the central nervous system (e.g., antipsychotics); and pregnancy/breastfeeding. The results of the

screening process and a gynecological examination (see below) formed the basis of inclusion or exclusion of women with PVD. For control women, the main inclusion criterion was the absence of chronic pain.

Measures

Gynecology exam. Because vulvodynia is a diagnosis of exclusion, the study gynecologist ruled out factors associated with vulvar pain (e.g., infections) in women with PVD. The gynecologist visually and manually examined the internal and external genitalia and reproductive organs and performed the cotton-swab test of the external genitalia (i.e., labia majora and minora, vestibule, perineum)—the main gynecological test for the diagnosis of PVD (Goldstein et al., 2016). The cotton-swab test consisted of the palpation of the labia majora, inner labia minora, midline areas, and six randomly order sites at the vestibule (e.g., 1 o'clock, 4-5 o'clock, 6 o'clock, etc.). After each palpation, the participant was asked to rate the pain on the intensity scale ranging from a rating of 0 (no pain at all) to 10 (worst pain ever felt). A mean average pain intensity rating of at least 1/10 during the cotton-swab test of the vestibule was required of women with PVD. If women did not meet criteria, they were excluded from the study and referred appropriately.

Quantitative sensory testing (QST) and sham MRI. Both the Quantitative Sensory Testing session and the sham MRI session were conducted in the Sham MRI room at the Queen's MRI Facility. The Sham MRI room is a mock-up of the actual MRI environment that provides the participant with the experience of undergoing MRI, without the magnetic field. Participants performed practice runs in order to become more familiarized with the environment and QST procedures, with the goal of ideally reducing their anxiety and providing more consistent fMRI results across repeated runs. Four participants in the PVD group completed the

QST training session and were therefore familiar with the heat stimulus paradigm but were unable to complete the sham within the mock MRI due to time constraints.[1] The QST session was devoted to determining a level of temperature that was considered "moderately painful" on the 101-point scale with verbal descriptors in increments of 10 (i.e., no sensation, warm, a barely painful sensation, very weak pain, weak pain, moderate pain, slightly strong pain, strong pain, very strong pain, nearly intolerable pain, intolerable pain) by each participant prior to the brain imaging session. If participants did not rate their pain intensity as "moderately painful" before reaching the safety limitation temperature (i.e., 51°C), 51°C was used to deliver heat pain for the safety of the participant. During the sensory testing session, the participant's heel of their thumb (Figure 1) rested on the Robotic Thermal Stimulator-1 (RTS-1; Figure 2), an MRI-compatible robotic contact heat thermal stimulator, made by Spinal Map Inc., Kingston.

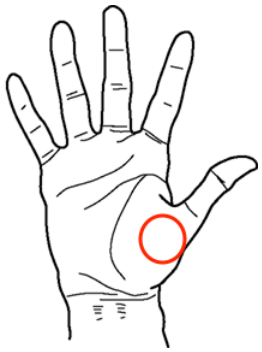

Figure 1. Stimulation Location at the Thenar Eminence

[1]SEM analysis of brainstem/spinal cord data excluding these four participants was run and no notable differences were found.

Figure 2. Robotic Thermal Stimulator 1 (RTS-1)

A heat thermode rose to contact the skin and lowered back into the box. The specifics of the contact such as duration and temperature were controlled by custom-made software installed on a laptop. The first three contacts lasted for a duration of 1.5 seconds at 45 °C. Participants were asked to give their ratings of the sensations on a 101-point scale of the intensity of the sensation, where 0 indicated "no sensation" and 100 indicated "intolerable pain"). After two minutes of rest to avoid sensitization, this process was repeated with temperatures of 46 °C and 47 °C. The purpose of this heat task was to train participants to rate their pain experience. Following this protocol, participants responded with their pain ratings to 10 consecutive, fixed heat pulses. After each of the four sets of pulses, participants were given two minutes to rest and rated any persisting pain at 15 seconds and 30 seconds prior to the last contact (Figure 3). Each set of pulses were done using a different temperature: 46 °C, 50 °C, 44 °C, and 48 °C. This protocol provided us with a measure of their pain sensitivity to use during the fMRI task, temporal summation of pain, and after sensations. The QST and Sham session took approximately 1 hour.

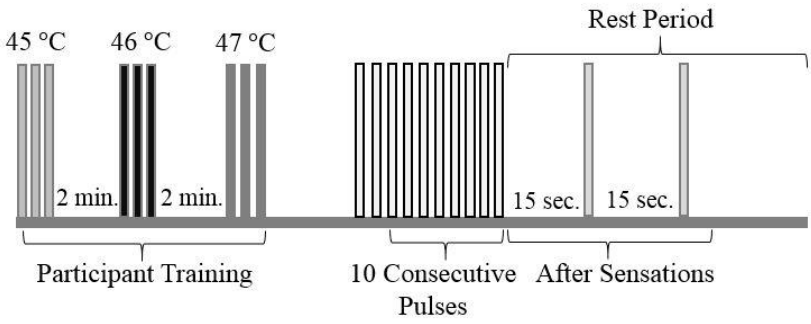

Figure 3. QST Training Design

Magnetic Resonance Imaging. Both the brain imaging session and CC/B imaging session were performed separately in order to avoid participant fatigue and the development of pain sensitivity. Imaging was carried out in a research-dedicated 3 tesla Siemens Magnetom Trio. Each imaging session took 1.5 hours and involved one anatomical scan and ten 4.5-minute functional scans, guided by initial localizer scans. Participants were positioned inside the MRI system, lying on their back, with padding and blankets for comfort and to prevent excessive movement. Posterior head, neck, and spine receiver coils were in place for detecting the MRI signal, and a mirror was positioned so that participants could view a rear-projection screen where the pain rating scale and instructions were displayed. Anatomical images of the CC/B were first acquired using a proton density weighted fast spin echo with 1 mm x 1 mm in-plane resolution and spanned from below the T1/T2 intervertebral disc to above the corpus collosum, in sagittal slices. Brain anatomical images were acquired using T1-weighted 3D sequence with 1mm x 1 mm resolution spanning the entire brain.

In order to obtain high quality images with good spatial fidelity, functional MR image data for CC/B was acquired from the same region as the anatomical scans with a HASTE (half-fourier single-shot fast spin-echo) sequence as per our established spinal cord fMRI method (Bosma & Stroman, 2014). This method has been demonstrated to provide T2-weighted blood

oxygenation level-dependent sensitivity, 1.5 mm x 1.5 mm x 2 mm resolution, and optimal image quality in the spinal cord and brainstem (Bosma & Stroman, 2014; Harita & Stroman, 2017; Bosma et al., 2015; Bosma et al., 2016). HASTE imaging acquisitions have been shown to be less sensitive to field inhomogenieties caused by the spinal cord than EPI acquisitions, which are prone to spatial distortions. Functional MRI data for the brain was obtained with a Gradient Echo EPI, providing us with 3.3 mm cubic resolution. During each acquisition of the CC/B or brain, participants were informed that a new run was about to begin but did not initially know if they would feel a painful stimulus or not. They were informed one minute after the start of acquisition whether they would feel heat. If they were to feel a stimulus, 10 heat pulses consisting of their moderate level of pain began to be applied to the heel of the thumb two minutes following the start of acquisition for 30 seconds (Figure 4). After exposure to the noxious stimulus, participants rested during 2 minutes of data acquisition, totaling 4.5 minutes of acquisition. If no noxious stimulus was to be applied, data acquisition continued while the participants rested. Participants were trained to rate their pain for each heat pulse mentally and reported the ratings for the first and last contacts following the run. If participants began to rate their pain as less than "moderately" painful or in the "unbearable" range during the MRI session (either after leaving the sham room, between MRI sessions, or across the MRI session runs), the researcher would again titrate the temperature to achieve a moderately painful stimulus. There were a total of 5 runs for each stimulus type and they were in a pseudo-randomized, counterbalanced order with 2-minute breaks to avoid sensitization of the skin. Each fMRI session, one imaging the brain and one imaging the CC/B, took 1.5 hours.

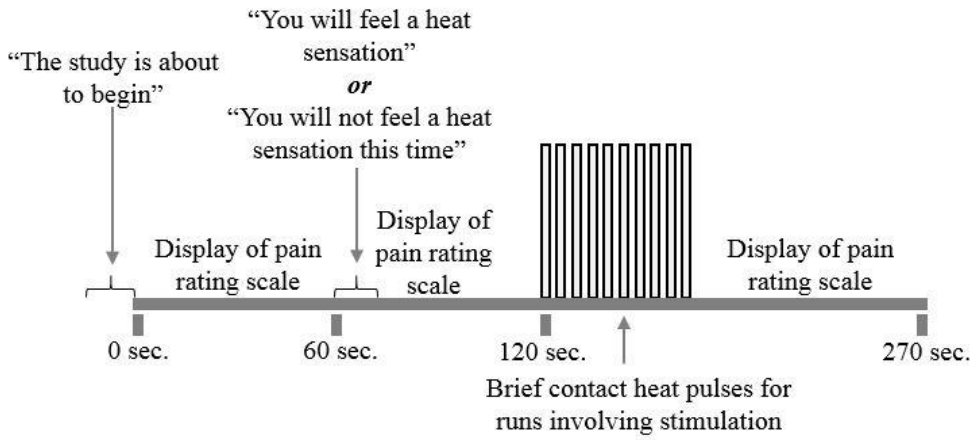

Figure 4. fMRI Study Design

Procedure

This study received ethical approval from the Health Sciences Research Ethics Board at Queen's University (Appendix A). Women with PVD found to be "initially" eligible based on the screening were scheduled for a gynecological examination to confirm their eligibility. Informed consent was obtained from women with PVD at their gynecological exam and control women gave informed consent at their first MRI session. Participants were asked to refrain from alcohol for at least 12 hours prior to the imaging portions of the study, and from caffeine for at least 6 hours before the study because of the effects these substances may have on mood, alertness, and the BOLD response. They were also asked to ensure that prior to the study they eat a regular meal at their usual meal time. Participants answered a series of questions and validated survey measures using Qualtrics, an online survey platform, on their own over an internet connection from home.

Before the first imaging session, a quantitative sensory testing/sham MRI session was conducted to establish the participant's pain sensitivity rating to be used during imaging tasks and familiarize them with the 100-point pain rating scale. The sham session was a means to acclimate participants to the imaging environment and train them in the study procedure. Before

entering the imaging environment, participants again confirmed that they had no contraindications for the subsequent magnetic resonance imaging, such as non-removable body piercings. If any item of clothing possibly contained materials that were not MRI safe, participants were asked to change into MRI-safe clothing provided by the facility. Participants were led from the sham MRI room where they were placed in the MRI with blankets and foam pillows to assure their comfort and minimize movement. They then returned for the second MRI session with identical procedures to the first. Participants were asked to retrieve any items that they were unable to bring into the room with the MRI and were then debriefed and compensated. Women with PVD were compensated 75 dollars and control participants 60 dollars, because PVD women also were required to attend the gynecological exam. Each session was randomized in a counter-balanced fashion for either brain or spinal cord imaging. This study was part of a larger study that involves saliva collection and the administration of validated measures; although these components will not be discussed in this thesis, information related to these components are included in Appendix G.

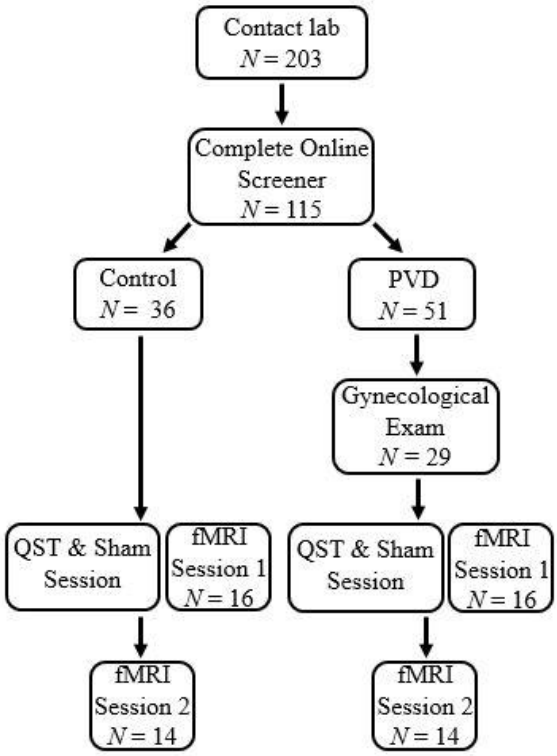

Figure 5. Participant Flow Chart

Sample Characteristics

In order to evaluate any differences between women with PVD and healthy control women, t-tests were used for continuous variables and Chi-Square analyses were used for categorical variables (Table 1). There were no significant differences between the groups on any of the demographic variables.

Table 1. *Participant Demographic Information*

	PVD Sample *n* = 16	Control Sample *n* = 16	Total Sample N = 32	*p*-value
Age (*M* (*SD*))	30.1 (9.8)	30.0 (9.7)	30.0 (9.6)	.765
Sexual Orientation (*n* (%))				.206
Heterosexual	16 (100.0)	12 (75.0)	28 (87.5)	
Same sex attracted		1 (6.3)	1 (3.1)	
Bisexual		2 (12.5)	2 (6.3)	
Not sure		1 (6.3)	1 (3.1)	

Relationship Status (*n* (%))				.743
Single (not dating)	1 (6.3)	2 (12.5)	3 (9.4)	
Casual sex (one partner)	1 (6.3)	1 (6.3)	2 (6.3)	
Casual sex (multiple partners)	1 (6.3)	2 (12.5)	3 (9.4)	
Dating partner (regularly)	2 (12.5)	2 (12.5)	4 (12.5)	
Dating partner (long distance)	1 (6.3)	2 (12.5)	3 (9.4)	
Living with partner	1 (6.3)	2 (12.5)	3 (9.4)	
Married	7 (43.8)	2 (12.5)	9 (15.6)	
Common-law	2 (12.5)	3 (18.8)	5 (15.6)	
Birthplace (*n* (%))				.283
Canada	13 (81.3)	12 (75.0)	25 (78.1)	
United States	1 (6.3)	1 (6.3)	2 (6.3)	
Europe		2 (18.8)	3 (3.1)	
Latin/South America	2 (12.5)		2 (6.3)	
Ethnicity (*n* (%))				.218
North American Aboriginal	1 (6.3)		1 (3.1)	
Canadian	14 (87.5)	10 (62.5)	24 (75.0)	
European	12 (75.0)	6 (37.5)	18 (56.3)	
Arab		1 (6.3)	1 (3.1)	
Asian		3 (18.8)	3 (9.4)	
Other	2 (12.5)		2 (6.3)	
Education (*n* (%))				.562
High school (complete)	2 (12.5)		2 (6.3)	
College/undergraduate degree (some)	3 (18.8)	5 (31.3)	8 (25.0)	
College/undergraduate degree (complete)	6 (37.5)	6 (37.5)	12 (37.5)	
Graduate/professional (some)	2 (12.5)	1 (6.3)	3 (9.4)	
Graduate/professional (complete)	3 (18.8)	4 (25.0)	7 (21.9)	
Income (*n* (%))				.960
$0 – 9,999	2 (12.5)	1 (6.3)	3 (9.4)	
$10,000 – 19,999	2 (12.5)	2 (12.5)	4 (12.5)	
$20,000 – 29,999	1 (6.3)	2 (12.5)	3 (9.4)	
$30,000 – 39,999	1 (6.3)	2 (12.5)	2 (6.3)	
$40,000 – 49,999		1 (6.3)	1 (3.1)	
$50,000 – 59,999	2 (12.5)	1 (6.3)	4 (12.5)	
$60,000 +	6 (37.5)	6 (37.5)	12 (37.5)	

Note. Due to missing data, multiple responses, and rounding, not all percentages add up to 100.

Data Preparation

Data Processing. Both brain and spinal cord data included 5 runs in both the no stimulation and stimulation conditions. Statistical Parametric Mapping (SPM12) software was used for the preprocessing of brain data, including realignment for motion correction, slice-

timing correction, and spatial normalization (Wellcome Department of Imaging Neuroscience). Spinal cord processing was performed using custom software called spinalfmri8 described previously (Bosma et al., 2014). First, imaging data were converted to NIFTI format. Sagittal slices were co-registered to correct for bulk body motion using a 3D, non-rigid registration tool (MIRT; Myronenko, & Song, 2009; Myronenko et al., 2010). These co-registration motion parameters were later used to model bulk movement and reduce noise. The images were spatially normalized to fit an anatomical template previously defined by 365 people (Bosma et al., 2015; Stroman et al., 2016). To avoid variability in T1 weighting, the first two volumes were discarded for each time series. We accounted for physiological noise in spinal cord runs by recording peripheral pulse timed in synchronization with MRI procedures. Regions of white matter were used to model global noise, because we do not expect the signal in these regions to vary based on the study paradigm. All of these models of noise were fit to the data using GLM and subtracted from the measured data.

Structural Equation Modeling (SEM). Using k-means clustering, regions of interest were divided in seven subdivisions to reduce the number of comparisons than would be necessary for voxel-to-voxel comparisons. By dividing each region into subdivisions, we are able to use time series properties to group voxels with significant BOLD responses independently of nonresponsive voxels and those consisting mostly of noise. For spinal cord data, the clusters near the edge of CSF space were removed from analyses, as they are susceptible to movement. SEM was used to identify patterns of correlations among BOLD responses in different regions as defined by our SEM models and to account for variance using a predetermined anatomical model of regional interactions. SEM is beneficial for this study's purposes, because it allows us to examine numerous different sources of coinciding input signals to a specific region. A brain stem

and spinal cord model of known anatomical connections allows us to infer on the directionality of interactions; this model is based on anatomical connection and regions known to be related to pain processing (Millan, 2002; Mayer, Labus, Tillisch, Cole, & Baldi, 2015; Apkarian et al., 2005; Khan et al., 2015): thalamus, hypothalamus, periaqueductal gray matter, locus coeruleus, parabrachial nucleus, nucleus raphe magnus, nucleus gigantocellularis, nucleus tractus solitarius, dorsal reticular nucleus, and C6 right dorsal portion of the spinal cord. The brain SEM model included the amygdala, thalamus, posterior parietal cortex, prefrontal cortex, anterior cingulate cortex, insular cortex, and the primary and secondary somatosensory cortices. Figure 6 describes the SEM connectivity models for the spinal cord and brain respectively. In order to calculate the strength of each region's input contribution to a specified target region (i.e. linear weighting factors), SEM was carried out by means of GLM for each run and group type. The weighting factors were calculated for each network component – the component including the region receiving input and all of the source regions contributing input. We allowed the SEM weighting factors to dynamically vary by having time periods lapsing seven volumes. We shifted the start of the epoch in a sliding window method by one volume at a time. The SEM weighting factors were assigned to the center time point of the epoch for assessing results. In order to examine the results, the weighting factors of the SEM were allocated to the time point at the epoch center.

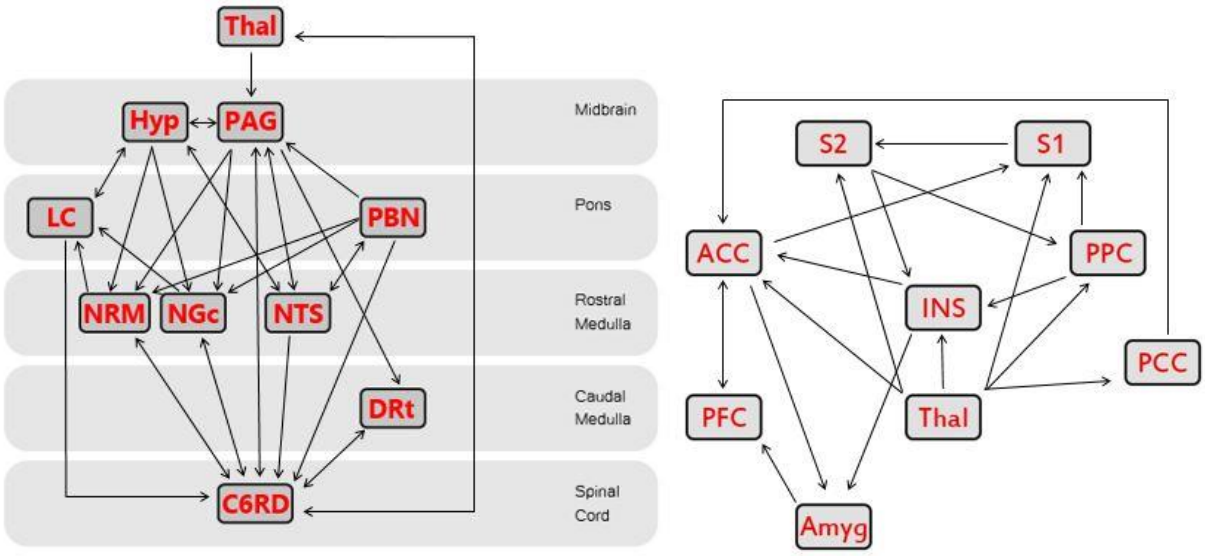

Figure 6. Spinal Cord/Brainstem and Brain SEM Models

Every combination of subdivisions (i.e., cluster) within each region were investigated to determine the best fit with the collected data. Therefore, the beta value for each connection within a network component was calculated multiple times with different combinations of source regions included. We calculated the goodness-of-fit by examining the proportion of the variance in the target region that was accounted for by the network component fit (i.e. R^2 value). We determined the probability of each fit occurring at random by converting R-values to Z-scores (Fisher's transform; $Z = \tanh^{-1}(R)\sqrt{tsize} - 3$) to estimate the significance of the fit. As additional confirmation, the fit was repeated by removing a single source region at a time from the network component to make sure all terms accounted for a significant portion of the variance in a target region. An F test was used to compare the original complete network to the reduced network using a cutoff F value corresponding to a $p < .05$. If any portion of the network did not account for a significant portion of the variance, it was not included in the results.

The significance of each total network component was determined by previously established probability distributions (Stroman, 2016). The distributions depend on model

parameters and the significance thresholds accounted for family wise error rate ($p_{fwe} < .05$), taking into consideration the number of network combinations across of subdivisions. The significance of β-values was inferred based on estimated standard error of the β-value, compared to the null hypothesis using a t-test. The complete best-fit network was identified for each run type for both PVD and control women by identifying the combinations of source regions that have the highest Z-values for each network component (i.e. the set of clusters with the highest Z-scores for each network component). The best-fit model explains the BOLD signal variations in the regions of interest based on known anatomical connections.

Significant differences in connectivity between regions were compared between study conditions using paired sample *t*-tests. Significant differences were inferred between two conditions if the *t*-test culminated in a Bonferroni-corrected $p < 0.05$, accounting for the total number of comparisons.

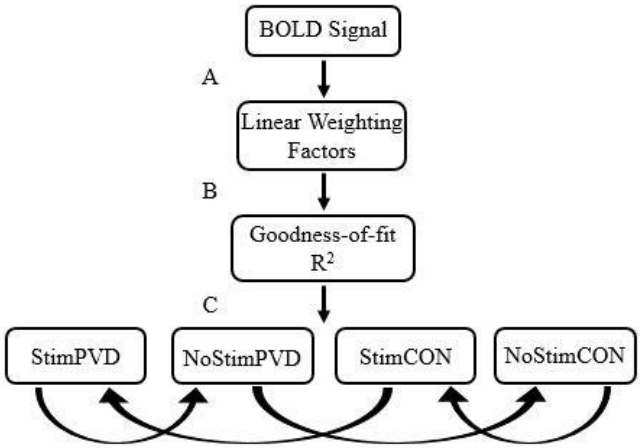

Figure 7. Summary of SEM and *t*-tests Data Reduction. Displaying (A) computation of linear weighting factors from collected BOLD response, (B) the calculation of the best model fit, and (C) *t*-tests comparing those connections between groups and conditions.

Bayesian Regression. SEM can describe temporal relationships between regions, but we also want to examine how the BOLD signals change over time in each region and observe the

systematic variations among individuals. Therefore, we used the subdivisions and regions defined by the SEM and used regressions to further investigate BOLD signal time courses. Bayesian regression allowed us to solve a linear approximation of our collected BOLD responses on an individual level, as predicted by the pain ratings and temperatures. The pain ratings and temperatures were centered so the mean value across all participants would equal zero. The function for approximating the BOLD responses (S_{BOLD}) includes the BOLD response at the average pain and temperature ratings (S_0), how the BOLD responses vary based on pain ratings (S_p) and temperature (S_t), and both squared temperature (S_{t2}) and pain ratings (S_{p2}) (Stroman et al., 2018):

$$S_{BOLD} = S_0 + S_p + S_t + S_{p2} + S_{t2}$$

Chapter 3

Results

QST Results

The pain ratings and temperature results from the MRI sessions are included in Table 2. Temperatures for the MRI sessions are displayed as the group averages of the individual participant average temperatures used during the MRI sessions and their corresponding pain ratings. Women with PVD had numerically lower moderately painful temperatures used during their MRI session than control women; however, this difference in temperature was not statistically significant. Differences in pain ratings were also not statistically significant.

Table 2. *Average Temperatures and Pain Ratings during MRI sessions*

	PVD Sample $n = 16$	Control Sample $n = 16$	*p*-value
Average Temperature	48.9 (2.0)	49.7 (1.1)	.281
Average Pain Ratings	48.4 (9.2)	48.5 (8.5)	.571

Spinal Cord and Brainstem SEM Results

Group Level Results. Group level connectivity was assessed to measure the significant best-fit model for each group. Figures 8-10 display the network of significant region connectivity for each group and condition before, during, and after painful stimulation. Table 3 summarizes the β values of significant group results for both the stimulation and no stimulation conditions. When the data was analyzed at the group level, there were no significant group results for control women during runs with no stimulation; therefore, there is no figure for control participants during this condition. The finding of non-significance indicates that there was no consistent connectivity across the group during the NoStim condition. During runs with stimulation for the

group level results, we can observe that control women consistently displayed greater consistency in connectivity between regions than women with PVD. Significant connectivity for control women was dominated by connections deriving from the hypothalamus and PAG, including the interconnectivity between both regions, which was found across the entire pain stimulus paradigm for controls but not for participants with PVD. There was only significant connectivity from the source region C6 and hypothalamus for women with PVD, specifically to the NGc and NRM.

Table 3. *Significant group Spinal Cord Connectivity between Regions for Both Stimulation and No Stimulation conditions*

Connectivity During No Stimulation Condition			
Paradigm period	Group	Region Source→Target	β
Before Stimulation	PVD	C6 right dorsal→ NGc	0.09 ± 0.03
During stimulation	PVD	PAG → NRM	0.40 ± 0.12
Connectivity During Stimulation Condition			
Before Stimulation	Control	PAG → Hypothalamus	0.48 ± 0.08
		PAG → NTS	0.40 ± 0.13
		Hypothalamus → PAG	0.57 ± 0.08
		Hypothalamus → LC	0.30 ± 0.08
		Hypothalamus → NGc	0.37 ± 0.11
During Stimulation	Control	PAG → Hypothalamus	0.58 ± 0.07
		PAG → NGc	0.64 ± 0.09
		PAG → NTS	0.63 ± 0.15
		Hypothalamus → PAG	0.83 ± 0.06
		Hypothalamus → LC	0.26 ± 0.08
		Hypothalamus → NGc	0.52 ± 0.10
		Thalamus → PAG	0.12 ± 0.03
		NTS → Hypothalamus	0.33 ± 0.07
		NGc → LC	0.21 ± 0.06
During Stimulation	PVD	C6 right dorsal→ NRM	-0.13 ± 0.04
After Stimulation	Control	PAG → Hypothalamus	0.69 ± 0.06
		PAG → NGC	0.54 ± 0.09
		Hypothalamus → PAG	0.89 ± 0.07
		Hypothalamus → NGC	0.46 ± 0.11
		Hypothalamus → NTS	0.41 ± 0.12
		NGc → LC	0.40 ± 0.06
		PBN → NGc	0.41 ± 0.11
		PBN → NTS	0.37 ± 0.10

After Stimulation	PVD	Hypothalamus → NRM	0.36 ± 0.10

Notes. DRt = dorsal reticular nucleus; LC = locus coeruleus; NGC = nucleus gigantocellularis; NRM = nucleus raphe magnus; NTS = nucleus tractus solitarius; PAG = periaqueductal grey; PBN = parabrachial nucleus.

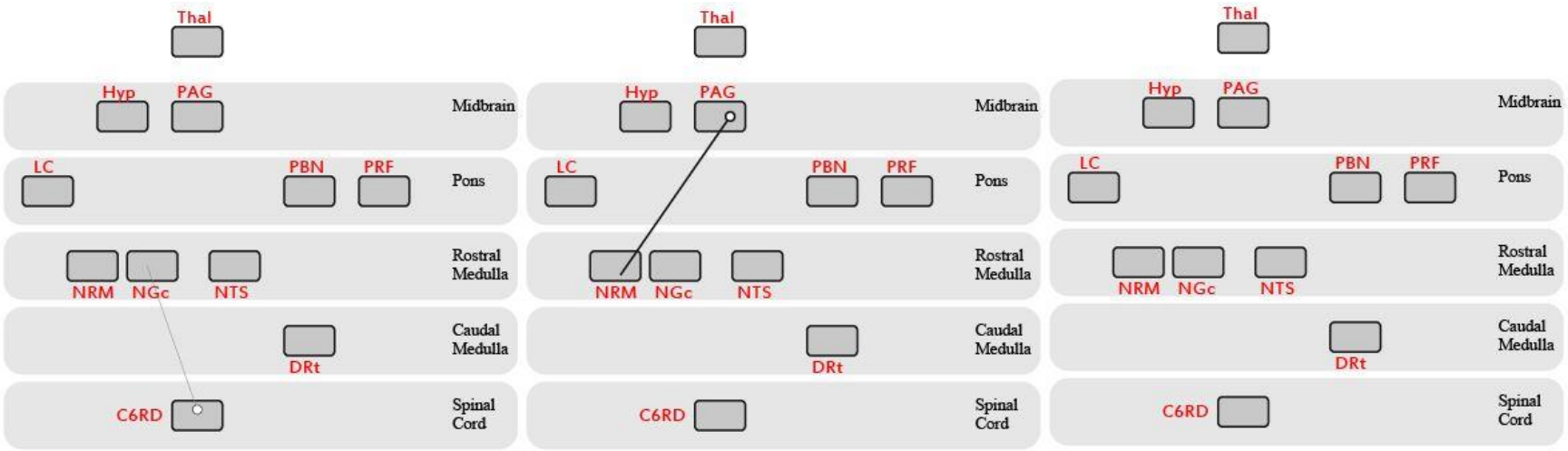

Figure 8. Spinal Cord/Brainstem Group Level NoStimPVD Dynamic SEM Results. Average group level connectivity values for women with PVD in the no stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

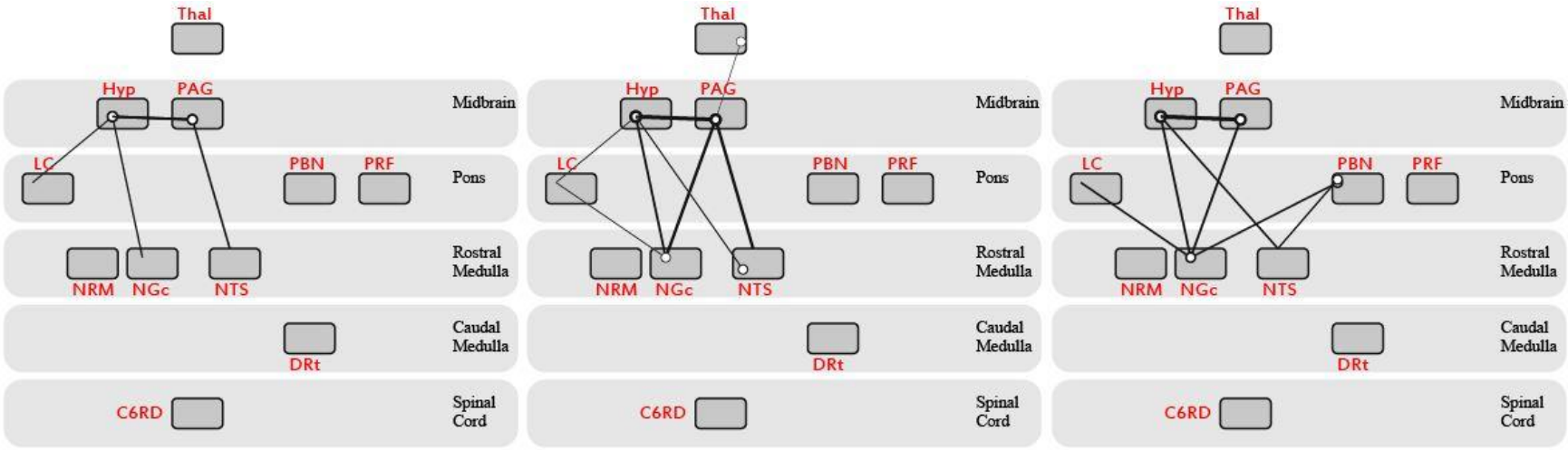

Figure 9. Spinal Cord/Brainstem Group Level StimCON Dynamic SEM Results. Average group level connectivity values for control participants in the stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

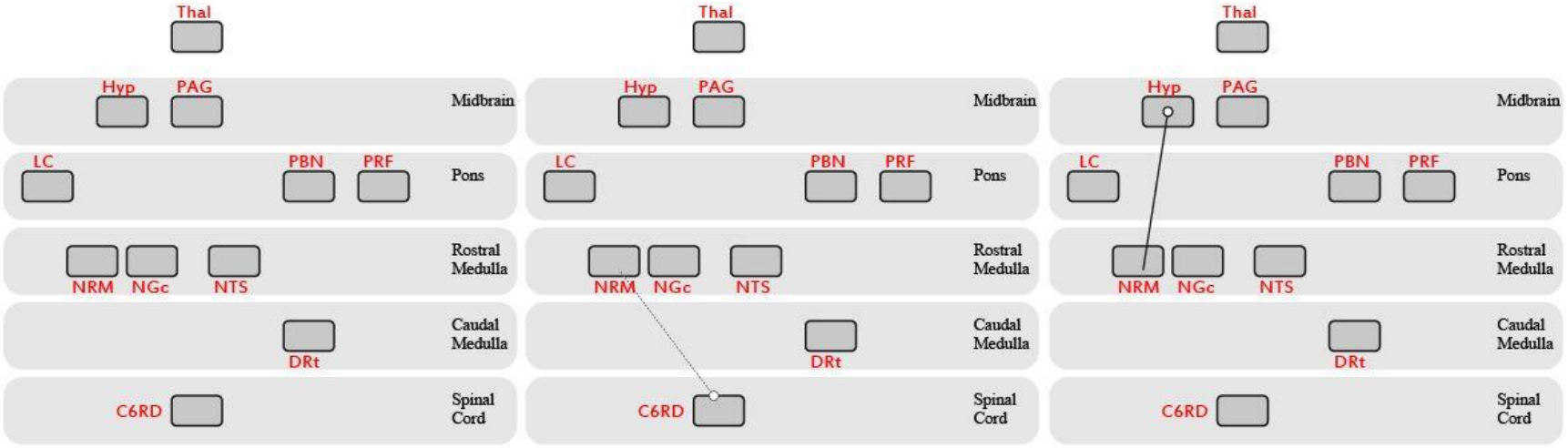

Figure 10. Spinal Cord/Brainstem Group Level StimPVD Dynamic SEM Results. Average group level connectivity values for women with PVD in the stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

Of those regions in the connectivity networks that accounted for the greatest variance, we performed a paired *t*-test to examine whether those identified connections were significantly different from one another between controls in the pain condition (StimCON), controls in the no pain condition (NoStimCON), women with PVD in the pain condition (StimPVD) and women with PVD in the no pain condition (NoStimCON). Significant differences among network connections were found when comparing all conditions and are summarized in Table 4.

Results revealed that, again, many of the significant differences in connectivity involved the PAG and hypothalamus. Connectivity originating from the cord was found to be significantly different when comparing women with PVD in the stimulation versus no stimulation condition, as well as when they were compared to control participants receiving stimulation. Both during and after stimulation, connections from the PAG to the NTS were significantly different between the NoStimCON group and the NoStimPVD group. When comparing control and PVD participants receiving stimulation, the beta weights for control participants were consistently higher than those for the PVD participants—and, in fact, PVD participants had reverse signs in seven of the 17 regions identified as being significantly different between these groups. This pattern was not found during the no stimulation conditions. When considering women with PVD receiving stimulation in comparison to no stimulation, only connectivity from the C6 right dorsal to the thalamus was found to be significantly different during stimulation. This finding is in stark contrast to the control group who had 13 significantly different connections across the paradigm periods.

Table 4. *Summary of Differences in Spinal Cord/Brainstem connectivity Networks between All Groups*

Paradigm Period	Region Source→Target	β StimCON	β NoStimCON	β StimPVD	β NoStimPVD
StimCON vs NoStimCON differences					
Before Stimulation	PAG → Hypothalamus	0.48 ± 0.08	0.11 ± 0.07	0.08 ± 0.07	0.18 ± 0.07
	Hypothalamus → PAG	0.60 ± 0.08	0.19 ± 0.12	0.15 ± 0.13	0.34 ± 0.12
During Stimulation	PAG → Hypothalamus	0.58 ± 0.07	-0.00 ± 0.09	0.15 ± 0.09	0.13 ± 0.08
	Hypothalamus → PAG	0.82 ± 0.05	0.00 ± 0.09	0.22 ± 0.10	0.21 ± 0.11
	NGC → LC	0.21 ± 0.06	-0.08 ± 0.08	0.02 ± 0.08	-0.15 ± 0.08
	Hypothalamus → NGC	0.52 ± 0.10	-0.04 ± 0.12	-0.07 ± 0.13	0.09 ± 0.15
	PAG → NGC	0.64 ± 0.09	0.01 ± 0.13	0.12 ± 0.12	-0.09 ± 0.12
	PAG → NTS	0.76 ± 0.10	-0.20 ± 0.15	0.14 ± 0.14	0.48 ± 0.16
After Stimulation	PAG → NTS	0.69 ± 0.06	0.07 ± 0.09	0.16 ± 0.09	0.17 ± 0.07
	PAG → Hypothalamus	0.87 ± 0.07	0.11 ± 0.10	0.26 ± 0.10	0.30 ± 0.12
	Hypothalamus → PAG	0.54 ± 0.09	-0.03 ± 0.12	0.32 ± 0.11	0.01 ± 0.11
	PAG → NGC	0.26 ± 0.08	-0.31 ± 0.16	0.05 ± 0.16	0.40 ± 0.13
	PAG → NTS	0.26 ± 0.08	-0.31 ± 0.16	0.05 ± 0.16	0.40 ± 0.13
	PAG → DRt	0.28 ± 0.04	-0.02 ± 0.10	0.08 ± 0.11	0.20 ± 0.12
StimPVD vs NoStimPVD differences					
During Stimulation	C6 right dorsal → Thalamus	0.36 ± 0.07	0.38 ± 0.07	0.02 ± 0.07	0.33 ± 0.07
NoStimCON vs NoStimPVD differences					
During Stimulation	PAG → NTS	0.76 ± 0.10	-0.20 ± 0.15	0.14 ± 0.14	0.48 ± 0.16
After Stimulation	PAG → NTS	0.26 ± 0.08	-0.31 ± 0.16	0.05 ± 0.16	0.40 ± 0.13
StimCON vs StimPVD differences					
Before Stimulation	PAG → Hypothalamus	0.48 ± 0.08	0.11 ± 0.07	0.08 ± 0.07	0.18 ± 0.07
	Hypothalamus → PAG	0.60 ± 0.08	0.19 ± 0.12	0.15 ± 0.13	0.34 ± 0.12
During Stimulation	C6 right dorsal → Thalamus	0.36 ± 0.07	0.38 ± 0.07	0.02 ± 0.07	0.33 ± 0.07
	PAG → Hypothalamus	0.58 ± 0.07	-0.00 ± 0.09	0.15 ± 0.09	0.13 ± 0.08
	Hypothalamus → PAG	0.82 ± 0.05	0.00 ± 0.09	0.22 ± 0.10	0.21 ± 0.11
	PAG → PBN	0.26 ± 0.03	0.13 ± 0.10	-0.03 ± 0.09	-0.02 ± 0.09

	Hypothalamus → NGC	0.52 ± 0.10	-0.04 ± 0.12	-0.07 ± 0.13	0.09 ± 0.15
	PAG → NGC	0.64 ± 0.09	0.01 ± 0.13	0.12 ± 0.12	-0.09 ± 0.12
	PAG → NTS	0.76 ± 0.10	-0.20 ± 0.15	0.14 ± 0.14	0.48 ± 0.16
	PAG → DRt	0.35 ± 0.04	0.16 ± 0.12	-0.03 ± 0.10	0.27 ± 0.11
After Stimulation	PAG→Hypothalamus	0.69 ± 0.06	0.07 ± 0.09	0.16 ± 0.09	0.17 ± 0.07
	Hypothalamus → PAG	0.87 ± 0.07	0.11 ± 0.10	0.26 ± 0.10	0.30 ± 0.12
	PAG → PBN	0.22 ± 0.03	0.17 ± 0.09	-0.07 ± 0.09	-0.03 ± 0.10
	Hypothalamus → NGC	0.46 ± 0.11	0.12 ± 0.12	-0.09 ± 0.12	0.29 ± 0.15
	PBN → NGC	0.41 ± 0.11	0.06 ± 0.12	-0.08 ± 0.12	-0.03 ± 0.10
	Hypothalamus → NTS	0.48 ± 0.10	0.21 ± 0.14	0.00 ± 0.13	0.14 ± 0.12
	C6 right dorsal → DRt	0.34 ± 0.08	0.20 ± 0.07	-0.00 ± 0.07	-0.02 ± 0.07

Notes. DRt = dorsal reticular nucleus; LC = locus coeruleus; NGC = nucleus gigantocellularis; NTS = nucleus tractus solitarius; PAG = periaqueductal grey; PBN = parabrachial nucleus.

Pain Correlation Results. During stimulation, there were significant correlations between pain ratings and connectivity strengths but in different connectivity regions for control (NTS→PBN) and PVD (PAG→HYP) groups.

Spinal Cord and Brainstem Bayesian Regression Results

Results of the spinal cord/brainstem Bayesian regression are displayed in Figures 11-14. During the period of time before and after the pain stimulation, all four groups significantly varied, or varied in relation to pain or temperature, in each region at some point. For this reason, during those paradigm periods we will specifically be focusing on those variations that appeared to differ in their pattern when comparing groups.

Between-group differences.

Before pain stimulation. Before the pain stimulation, both NoStimCON and NoStimPVD participants had a similar pattern of variation in their BOLD signal response in the NGC following the indication that they would feel pain and at the end of the indication period in the thalamus. Before pain began, participants in the StimPVD group showed significant variation which was not seen in the control participants. There was an inverse pattern in the PBN at the end of the period between the PVD conditions and the control conditions. All of the groups had a similar variation pattern in the NTS, but both of the PVD conditions had a larger amount of variation in BOLD signal response than the control groups. In the hypothalamus, the control participants not receiving pain exhibited significant variation when they were told what stimulation condition to expect, but this pattern was not seen in the NoStimPVD group. They were in fact inverse patterns near the condition indication.

During pain stimulation. During the period of time when the noxious stimulus was applied, the NRM had significant BOLD signal response variations in control participants and

the C6 significantly varied for PVD participants. During the corresponding period of time for the no stimulation condition, control participants significantly varied in their BOLD signal responses in the NTS and thalamus, whereas PVD participants varied in the DRt and PBN. Also, the BOLD signal variations in many regions scaled with pain ratings, such as the NRM, DRt, PAG, PBN, and hypothalamus for controls and the PAG, PBN, and C6 for PVD. During the no stimulation condition, variations in response scaled with pain ratings in the NTS for controls and PAG for women with PVD. BOLD signal responses were also analyzed to assess if variation scaled with temperatures used. The signal variations for StimCON scaled with temperature in the LC, PAG, and PBN, when in comparison StimPVD participants scaled with temperature in the PAG and C6. During the condition without a stimulus, only women with PVD showed variations that scaled with temperature in the LC and PAG. NoStimCON and NoStimPVD participants displayed inverse patterns in their NTS. In addition, the NGC showed larger variations in the StimCON group than StimPVD group.

After pain stimulation. After pain stimulation, the no stimulation condition for both groups had inverse variation patterns to one another in multiple regions, including the C6, DRt, LC, NGC, and PBN. BOLD signal variation in the NTS is similar between the PVD and control groups. In the C6, the NoStimPVD group showed inverse pattern variations to the other three groups following the completion of pain. Similarly, the NoStimCON group also had inverse variation after the end of the pain stimulation period to the three other groups in the NGC.

Condition differences. Before the pain stimulation, both PVD conditions had an inverse pattern of variation through the period preceding pain in the LC. During pain stimulation, StimCON and StimPVD participants appeared to have larger variation in the C6 during the stimulation period in comparison to the conditions with no stimulation, with PVD variation

patterns being slightly larger than controls. After pain stimulation, the stimulation conditions both showed greater variation in the DRt once the pain ended in comparison to the no stimulation conditions; however, the StimCON group appeared to have larger variation than the StimPVD group. The stimulation conditions showed variations in the NTS response patterns immediately following the end of pain that were not found in the no stimulation conditions.

Results related to group and condition. Before pain stimulation, the StimCON group had an inverse NTS variation pattern to the NoStimCON group immediately before the pain starts, which was not seen in the PVD conditions. In the PAG, the StimPVD group exhibited a similar pattern to both no stimulation groups, whereas the StimCON group had the inverse pattern to all three. During pain stimulation, there was not much significant NRM variation for NoStimCON participants; however, StimCON participants showed a decrease in the BOLD signal response. This difference between conditions was not found in the PVD group. In addition, during this period, the StimCON group did not display the same NTS variation as the NoStimCON participants, and StimPVD participants also had an inverse variation pattern to no stimulation runs. After pain stimulation, the control group displayed greater variation in their stimulation condition than in the no stimulation condition within the thalamus, which was not seen in the PVD group.

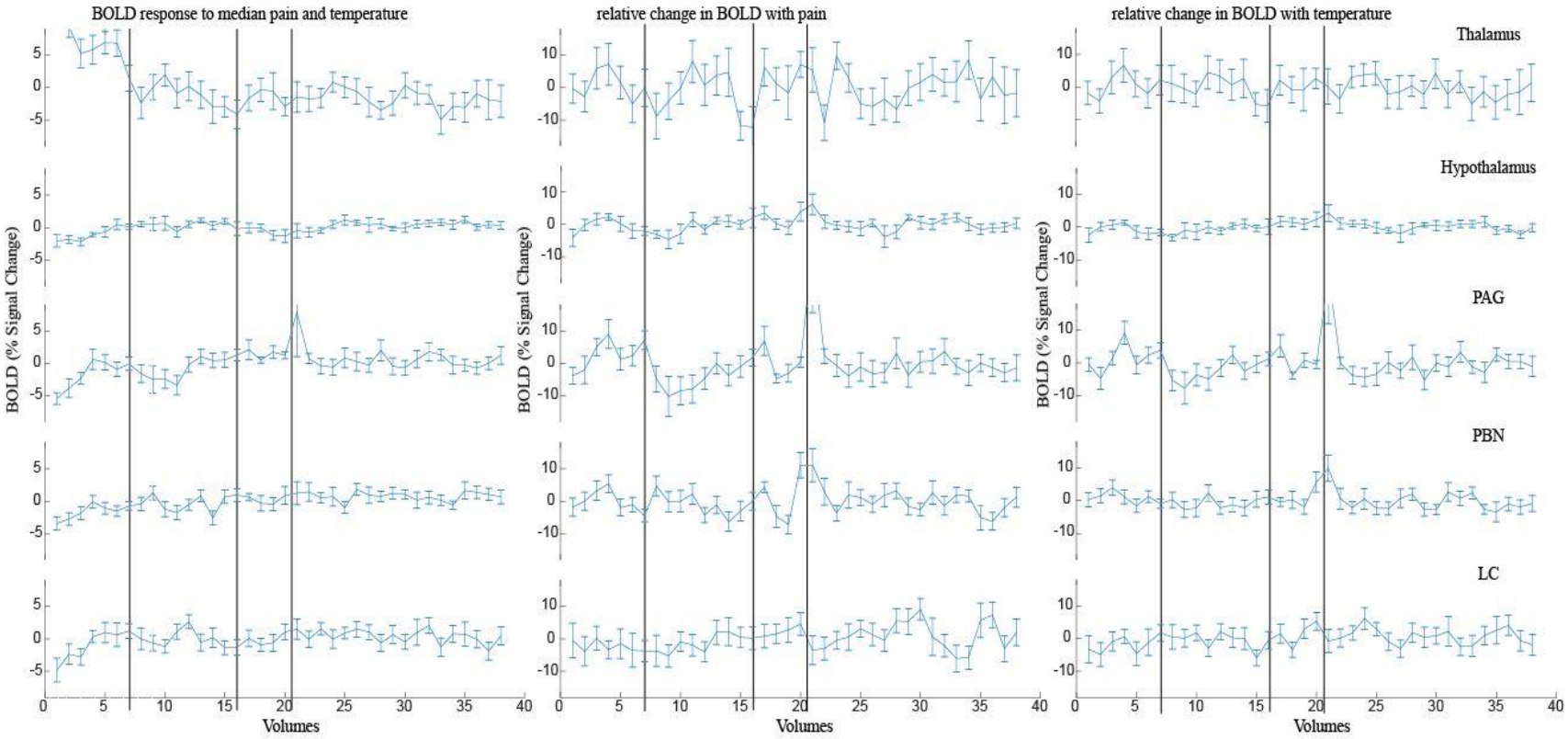

Figure 11. Spinal Cord/Brainstem StimCON Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

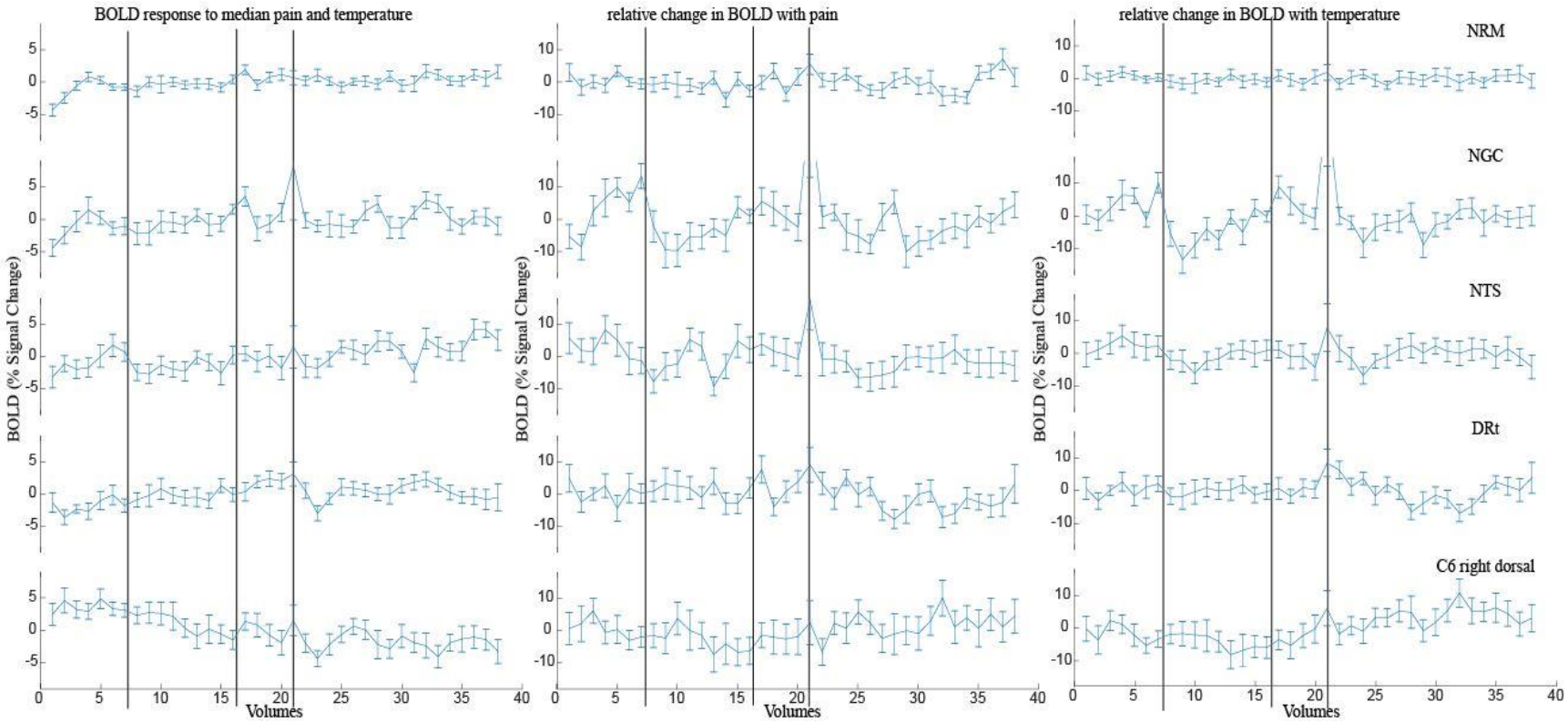

Figure 11. Spinal Cord/Brainstem StimCON Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

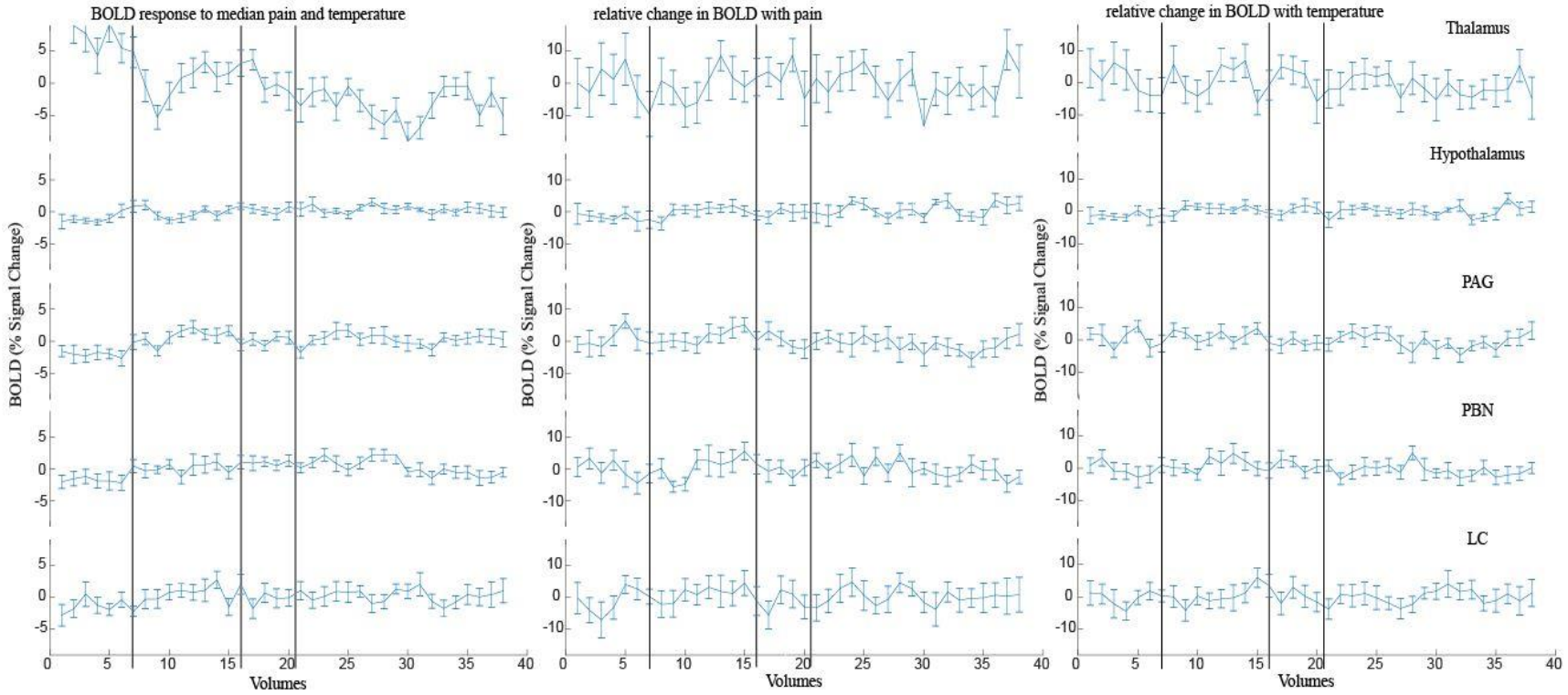

*Figure 12***.** Spinal Cord/Brainstem NoStimCON Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

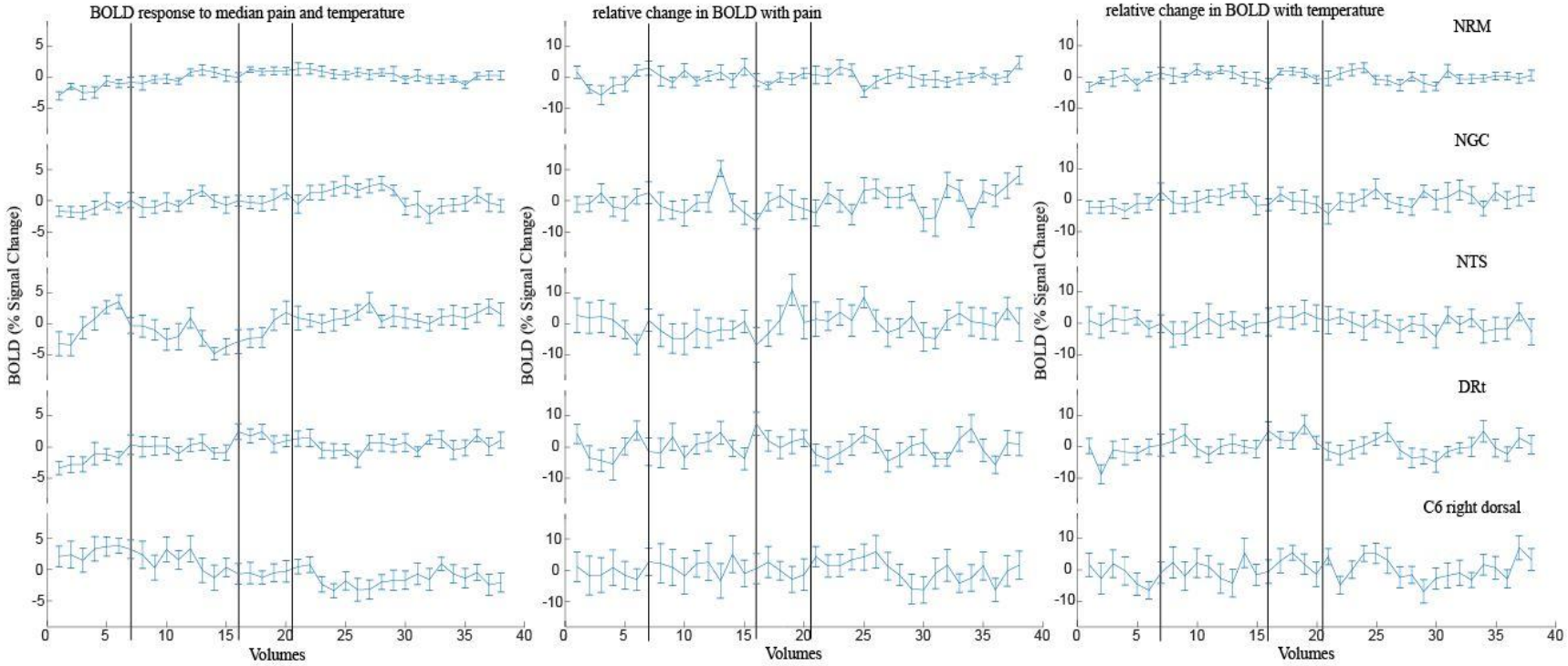

Figure 12. Spinal Cord/Brainstem NoStimCON Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

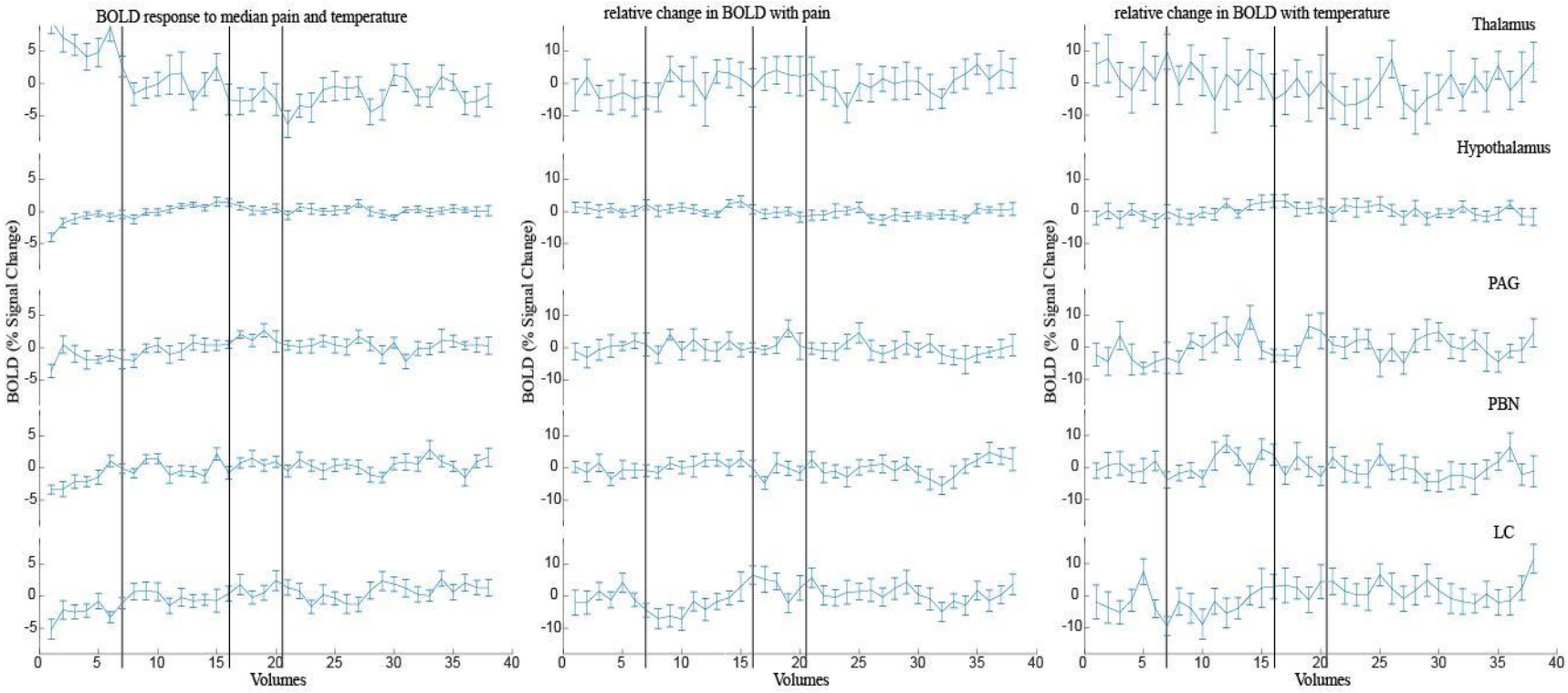

Figure 13. Spinal Cord/Brainstem StimPVD Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

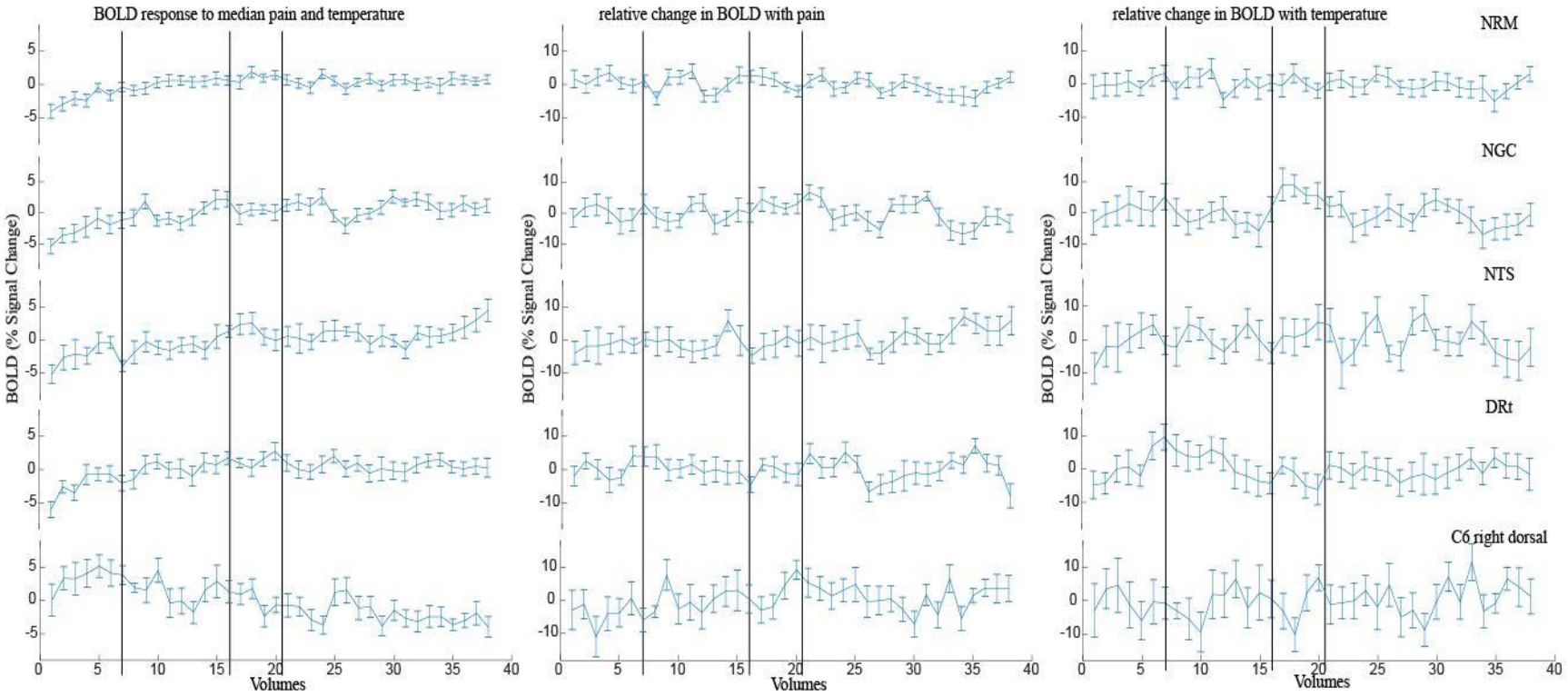

Figure 13. Spinal Cord/Brainstem StimPVD Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

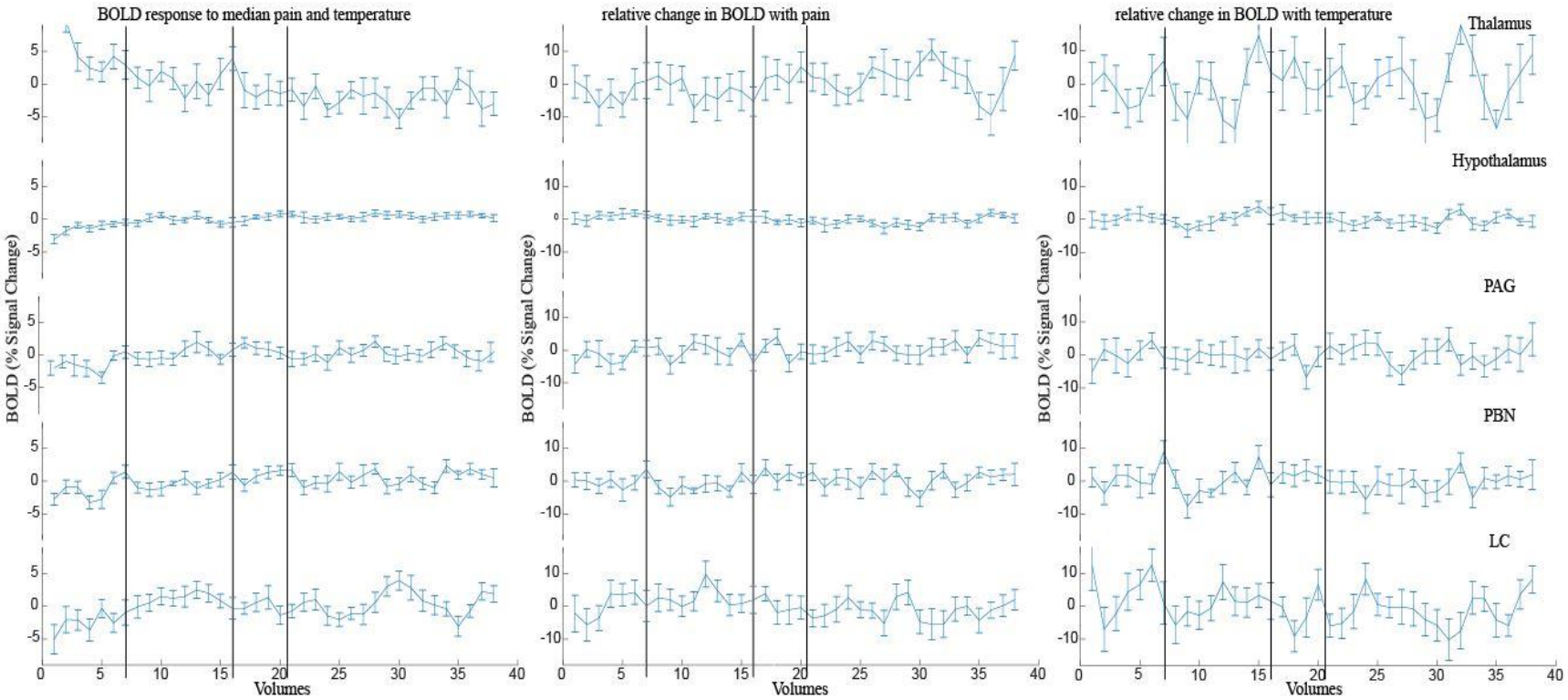

Figure 14. Spinal Cord/Brainstem NoStimPVD Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

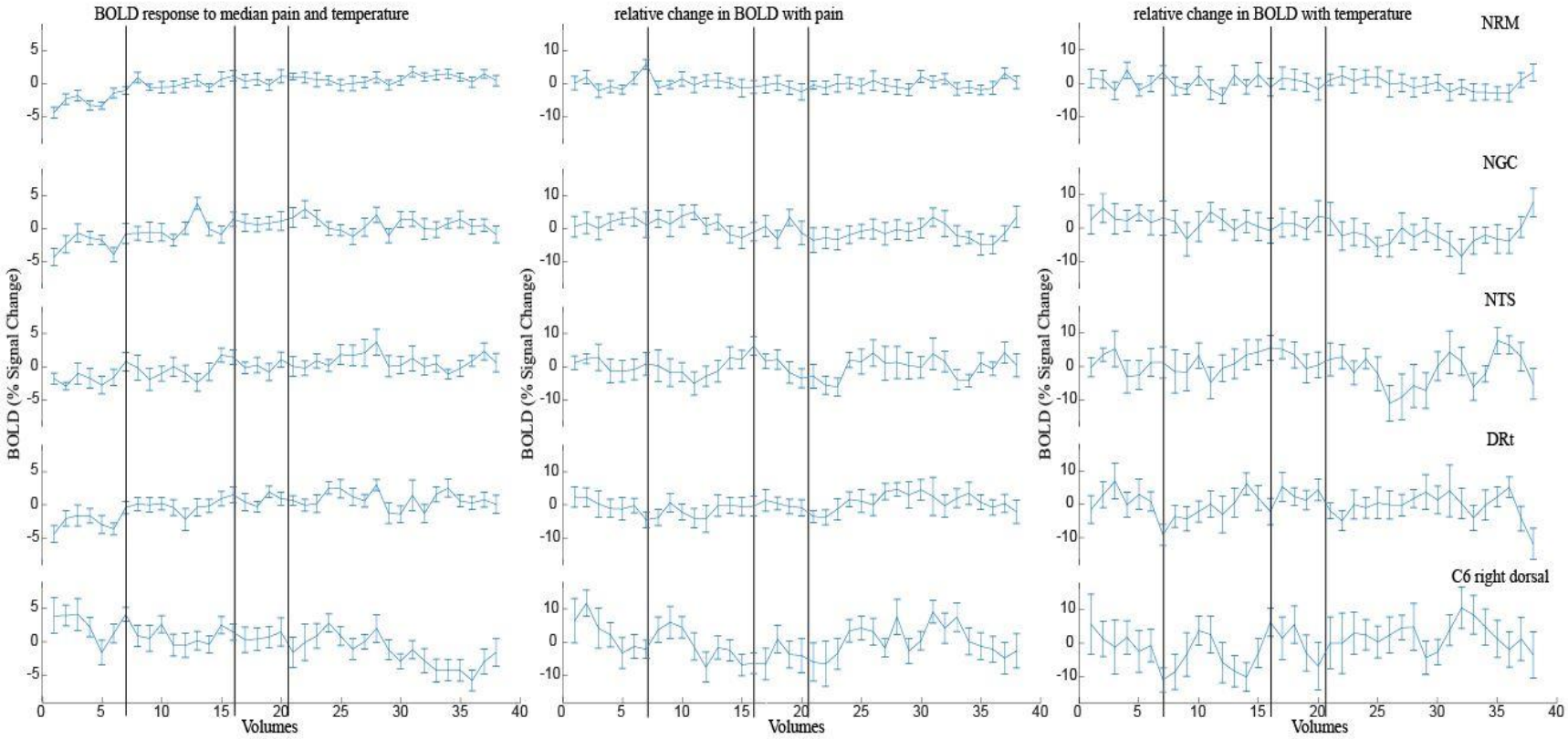

Figure 14. Spinal Cord/Brainstem NoStimPVD Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

Brain SEM Results

Group Level Results. Significant connectivity was found between regions across the entire study paradigm for both groups. Figures 15-18 display the network of significant region connectivity for each group and condition before, during, and after pain stimulation. Table 5 summarizes the β-values of significant connectivity for both groups and conditions. The connectivity networks for both groups were very similar. There was more consistent Amygdala → PFC and S2 → PPC connectivity in both the StimCON and NoStimCON groups across the time course than in women with PVD. Thalamus → S2 connectivity is seen in the NoStimCON participants but not in the StimCON participants; however, the opposite was true for PVD participants.

Table 5. *Significant Group Brain Connectivity between Regions for Both Stimulation and No Stimulation Conditions*

Connectivity During No Stimulation Condition			
Paradigm period	Region Source→Target	β PVD	β Control
Before Stimulation	INS → ACC	0.26 ± 0.06	0.25 ± 0.06
	ACC → PFC	-0.07 ± 0.02	0.35 ± 0.08
	ACC → S1	0.57 ± 0.11	0.44 ± 0.13
	PCC → ACC	0.43 ± 0.1	0.65 ± 0.10
	PPC → S1	0.45 ± 0.08	0.41 ± 0.09
	S1 → S2	0.33 ± 0.08	0.19 ± 0.05
	S2 → PPC	0.56 ± 0.08	0.36 ± 0.08
	Amygdala → PFC	-	0.58 ± 0.09
	Thalamus → INS	0.32 ± 0.08	0.38 ± 0.08
	Thalamus → ACC	1.15 ± 0.12	0.42 ± 0.07
	Thalamus → PCC	0.71 ± 0.12	1.23 ± 0.12
	Thalamus → S1	0.39 ± 0.08	0.69 ± 0.14
	S2 → INS	0.26 ± 0.07	-
During Stimulation	INS → ACC	-	0.24 ± 0.06
	ACC → PFC	-0.06 ± 0.02	-0.21 ± 0.07
	PCC → ACC	0.57 ± 0.10	0.67 ± 0.12
	PPC → INS	-	0.21 ± 0.05
	PPC → S1	0.32 ± 0.06	0.55 ± 0.07

	S1 → S2	0.35 ± 0.09	0.27 ± 0.06
	S2 → INS	-	0.29 ± 0.06
	S2 → PPC	0.56 ± 0.08	0.40 ± 0.07
	Amygdala → PFC	-	0.44 ± 0.08
	Thalamus → ACC	0.50 ± 0.07	0.31 ± 0.08
	Thalamus → PCC	0.81 ± 0.10	1.15 ± 0.10
	Thalamus → S1	0.52 ± 0.10	0.59 ± 0.13
After Stimulation	ACC → PFC	0.57 ± 0.11	-0.21 ± 0.07
	ACC → S1	-0.07 ± 0.02	0.37 ± 0.11
	PCC → ACC	0.50 ± 0.11	0.61 ± 0.11
	PPC → S1	0.42 ± 0.08	0.32 ± 0.07
	S1 → S2	0.31 ± 0.09	0.28 ± 0.05
	S2 → PPC	-	0.41 ± 0.10
	Amygdala → PFC	-	0.36 ± 0.08
	Thalamus → INS	-	0.34 ± 0.08
	Thalamus → ACC	0.40 ± 0.07	0.43 ± 0.09
	Thalamus → PCC	0.72 ± 0.12	0.88 ± 0.12
	Thalamus → S1	0.37 ± 0.11	0.74 ± 0.14
Connectivity During Stimulation Condition			
Before Stimulation	INS → ACC	0.17 ± 0.06	0.25 ± 0.06
	ACC → PFC	-0.09 ± 0.02	-0.35 ± 0.08
	ACC → S1	0.50 ± 0.11	0.44 ± 0.13
	PCC → ACC	0.46 ± 0.12	0.65 ± 0.10
	PPC → S1	0.53 ± 0.07	0.41 ± 0.09
	S1 → S2	0.30 ± 0.05	0.19 ± 0.05
	S2 → PPC	-	0.36 ± 0.08
	Amygdala → PFC	0.25 ± 0.06	0.58 ± 0.09
	Thalamus → INS	-	0.38 ± 0.08
	Thalamus → ACC	0.41 ± 0.08	0.42 ± 0.07
	Thalamus → PCC	1.15 ± 0.12	1.23 ± 0.12
	Thalamus → S1	0.46 ± 0.12	0.69 ± 0.14
	Thalamus → S2	-	0.33 ± 0.09
During Stimulation	INS → ACC	0.25 ± 0.06	0.24 ± 0.06
	ACC → PFC	-0.21 ± 0.06	-0.21 ± 0.07
	PCC → ACC	0.60 ± 0.10	0.67 ± 0.12
	PPC → INS	0.04 ± 0.01	0.21 ± 0.05
	PPC → S1	0.50 ± 0.04	0.55 ± 0.07
	S1 → S2	0.22 ± 0.04	0.27 ± 0.06
	S2 → INS	-	0.29 ± 0.06
	S2 → PPC	0.40 ± 0.12	0.40 ± 0.07
	Amygdala → PFC	0.31 ± 0.07	0.44 ± 0.08
	Thalamus → ACC	0.33 ± 0.08	0.31 ± 0.08
	Thalamus → PCC	0.73 ± 0.14	1.15 ± 0.10
	Thalamus → S1	0.43 ± 0.11	0.59 ± 0.13
	Thalamus → S2	-	0.51 ± 0.11

	ACC → S1	0.38 ± 0.08	-
	Thalamus → INS	0.40 ± 0.09	-
After Stimulation	INS → ACC	-	0.21 ± 0.06
	ACC → PFC	-0.13 ± 0.02	-0.30 ± 0.07
	PCC → ACC	-0.20 ± 0.07	0.71 ± 0.11
	PPC → INS	-	0.15 ± 0.05
	S2 → PPC	-	0.30 ± 0.09
	Amygdala → PFC	0.28 ± 0.06	0.24 ± 0.06
	Thalamus → INS	-	0.37 ± 0.09
	Thalamus → ACC	0.36 ± 0.08	0.27 ± 0.08
	Thalamus → PCC	1.05 ± 0.10	1.09 ± 0.11
	Thalamus → S1	0.64 ± 0.10	0.89 ± 0.13
	Thalamus → S2	0.34 ± 0.11	0.58 ± 0.10
	ACC → S1	0.65 ± 0.09	-
	PPC → S1	0.45 ± 0.08	-
	PFC → ACC	-0.20 ± 0.07	-
	S1 → S2	0.24 ± 0.06	-
	S2 → INS	0.18 ± 0.06	-

Notes. ACC = anterior cingulate cortex; INS = insular cortex; PCC = posterior cingulate cortex; PFC = prefrontal cortex; PPC = posterior parietal cortex; S1 = primary somatosensory cortex; S2 = secondary somatosensory cortex.

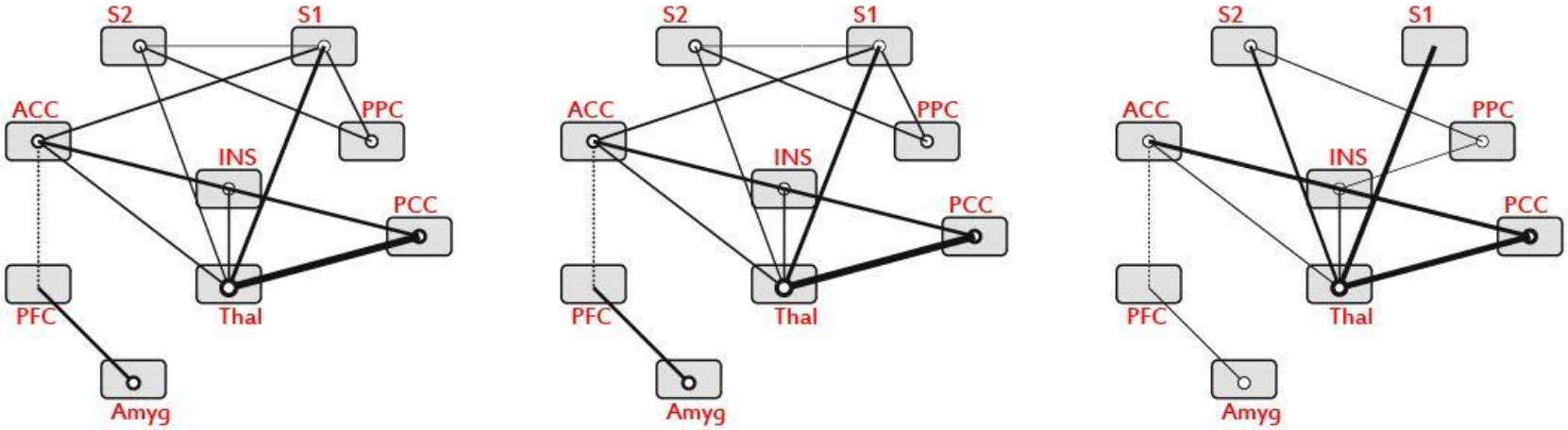

Figure 15. Brain Group Level NoStimCON Dynamic SEM Results. Average group level connectivity values for control participants in the no stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

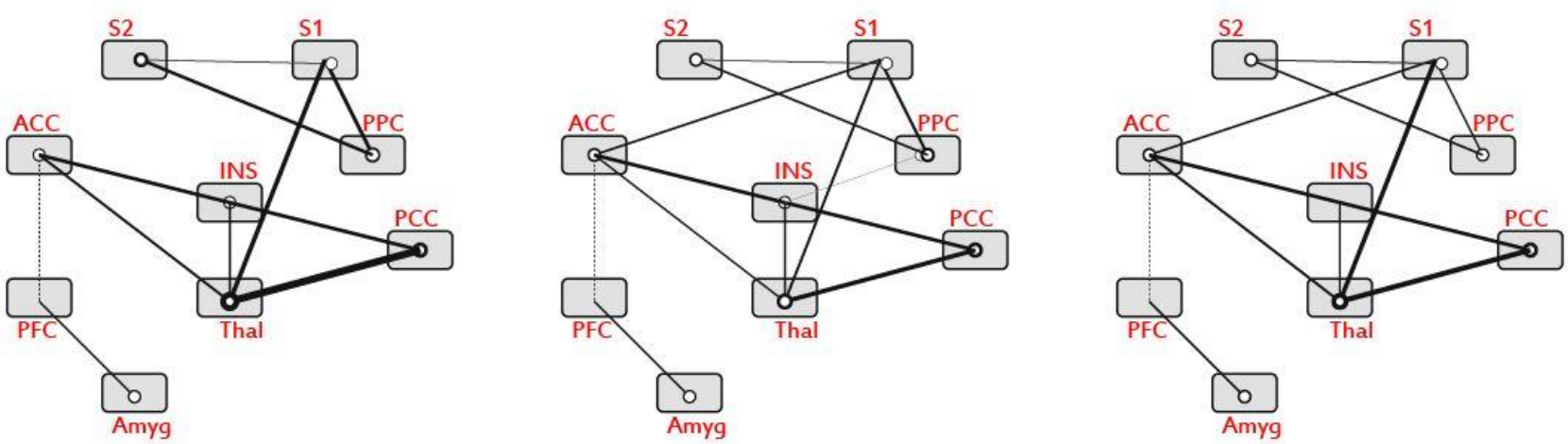

Figure 16. Brain Group Level StimCON Dynamic SEM Results. Average group level connectivity values for control participants in the stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

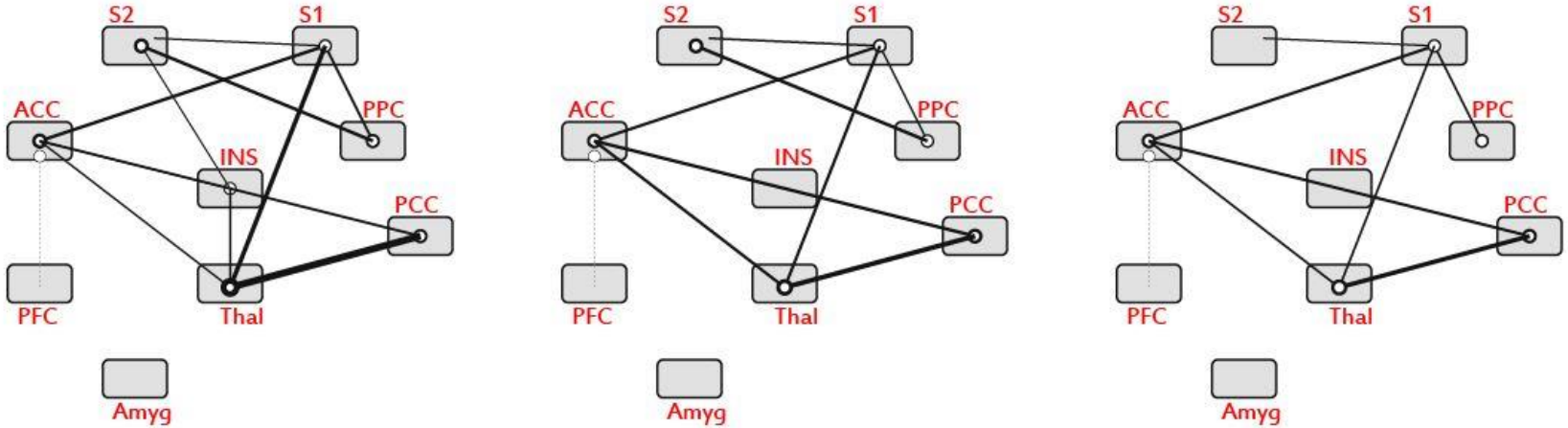

Figure 17. Brain NoStimPVD Dynamic SEM Results. Average group level connectivity values for women with PVD in the no stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

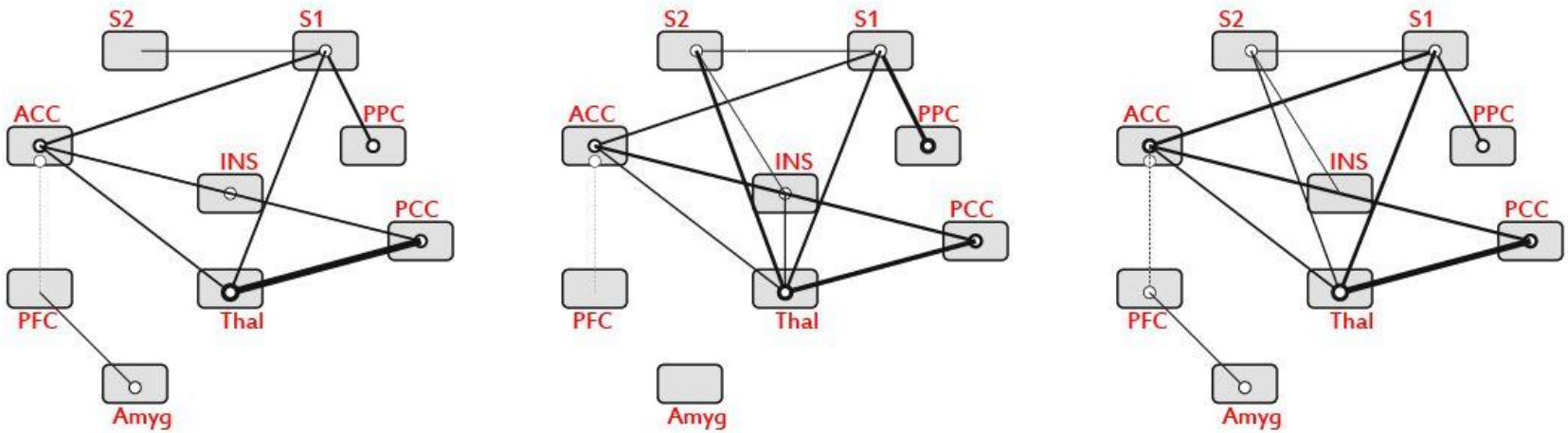

Figure 18. Brain StimPVD Dynamic SEM Results. Average group level connectivity values for women with PVD in the stimulation condition before (left), during (middle), and after (right) the pain stimulation period. Circles indicate where the connection derives from and the line ends in the target region. Line thickness indicates connectivity strength between regions and dashed lines represent negative values.

Significant differences among brain network connections were found when comparing all conditions, and these results are summarized in Table 6. When comparing the differences between stimulation conditions for each group, control participants had noticeably fewer significantly different connections than PVD participants. Only during the period of stimulation did control participants have significantly different connectivity from the ACC to amygdala and the thalamus to S1. Of the connectivity network components that were significantly different between PVD stimulation conditions, the thalamus, S1, and S2 made up a majority of the source and target regions. When comparing the StimCON and StimPVD groups, significant differences occurred before and during pain stimulation. The thalamus, S1, S2, and PPC also made up a large portion of the regions in significantly different connections between StimCON and StimPVD participants.

Table 6. *Summary of Differences in Brain Connectivity Networks between All Conditions*

Paradigm Period	Region Source→Target	β StimCON	β NoStimCON	β StimPVD	β NoStimPVD
StimCON vs NoStimCON differences					
During Stimulation	ACC → Amygdala	-0.06 ± 0.01	-0.01 ± 0.01	-0.03 ± 0.02	-0.03 ± 0.02
	Thalamus → S1	0.10 ± 0.01	0.06 ± 0.01	-0.03 ± 0.01	-0.04 ± 0.01
StimPVD vs NoStimPVD differences					
Before Stimulation	Thalamus → INS	0.38 ± 0.07	0.40 ± 0.07	0.08 ± 0.08	0.40 ± 0.08
	ACC → S1	0.18 ± 0.03	0.29 ± 0.03	0.08 ± 0.03	0.27 ± 0.04
	Thalamus → S1	0.07 ± 0.01	0.01 ± 0.01	-0.00 ± 0.01	-0.06 ± 0.01
	Thalamus → S2	0.37 ± 0.08	0.43 ± 0.07	0.82 ± 0.08	0.54 ± 0.06
During Stimulation	S2 → PPC	0.40 ± 0.07	0.39 ± 0.12	0.25 ± 0.05	0.55 ± 0.08
	PPC → S1	0.54 ± 0.07	0.49 ± 0.04	0.72 ± 0.07	0.32 ± 0.06
	Thalamus → S2	0.51 ± 0.11	0.29 ± 0.09	0.65 ± 0.10	0.22 ± 0.08
After Stimulation	INS → Amygdala	0.28 ± 0.07	0.22 ± 0.05	0.37 ± 0.09	0.07 ± 0.07
	PPC → S1	0.54 ± 0.07	0.49 ± 0.04	0.72 ± 0.07	0.32 ± 0.06
	Thalamus → S2	0.51 ± 0.11	0.29 ± 0.09	0.65 ± 0.10	0.22 ± 0.08

NoStimCON vs NoStimPVD differences					
Before Stimulation	INS → Amygdala	0.28 ± 0.05	0.27 ± 0.06	0.21 ± 0.08	-0.08 ± 0.08
	Thalamus → S1	0.07 ± 0.01	0.01 ± 0.01	-0.00 ± 0.01	-0.06 ± 0.01
	PPC → S2	0.76 ± 0.05	0.73 ± 0.05	0.25 ± 0.07	0.23 ± 0.07
	S1 → S2	-0.17 ± 0.05	-0.20 ± 0.04	0.05 ± 0.10	0.25 ± 0.08
StimCON vs StimPVD differences					
Before Stimulation	Amygdala → PFC	0.58 ± 0.08	0.32 ± 0.07	0.24 ± 0.05	0.16 ± 0.05
	Thalamus → ACC	-0.05 ± 0.01	-0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
	Thalamus → PCC	1.07 ± 0.02	1.05 ± 0.02	0.69 ± 0.03	0.68 ± 0.02
	S2 → PPC	1.07 ± 0.02	1.12 ± 0.02	1.18 ± 0.03	1.19 ± 0.03
	PPC → S1	0.55 ± 0.05	0.53 ± 0.05	0.80 ± 0.04	0.69 ± 0.05
	Thalamus → S1	0.07 ± 0.01	0.01 ± 0.01	-0.00 ± 0.01	-0.06 ± 0.01
	PPC → S2	0.76 ± 0.05	0.73 ± 0.05	0.25 ± 0.07	0.23 ± 0.07
	Thalamus → S2	0.37 ± 0.08	0.43 ± 0.07	0.82 ± 0.08	0.54 ± 0.06
	INS → Amygdala	0.22 ± 0.03	0.28 ± 0.03	0.36 ± 0.03	0.35 ± 0.04
	ACC → Amygdala	0.31 ± 0.03	0.26 ± 0.04	0.15 ± 0.04	0.18 ± 0.04
During Stimulation	Amygdala → PFC	0.44 ± 0.07	0.31 ± 0.07	0.07 ± 0.05	0.09 ± 0.06
	Thalamus → ACC	-0.05 ± 0.01	-0.05 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
	Thalamus → PCC	1.03 ± 0.02	1.08 ± 0.02	0.70 ± 0.03	0.66 ± 0.03
	S2 → PPC	0.97 ± 0.02	1.11 ± 0.02	1.13 ± 0.03	1.17 ± 0.03
	PPC → S1	0.41 ± 0.05	0.56 ± 0.04	0.79 ± 0.04	0.70 ± 0.04
	Thalamus → S1	0.10 ± 0.01	0.06 ± 0.01	-0.03 ± 0.01	-0.04 ± 0.01
	PPC → S2	0.76 ± 0.06	0.74 ± 0.05	0.21 ± 0.07	0.13 ± 0.08
	Thalamus → S2	0.39 ± 0.11	0.41 ± 0.07	0.97 ± 0.08	0.59 ± 0.07

Notes. ACC = anterior cingulate cortex; INS = insular cortex; PCC = posterior cingulate cortex; PFC = prefrontal cortex; PPC = posterior parietal cortex; S1 = primary somatosensory cortex; S2 = secondary somatosensory cortex.

Pain Correlation Results. Before stimulation, there were significant correlations between pain ratings and connectivity strengths but in different connectivity regions for control (S1→S2) and PVD (ACC→Amygdala, INS→Amygdala) groups.

Brain Bayesian Regression Results

Between-group differences. Figures 19-22 display the network of significant region connectivity for each group and condition before, during, and after pain stimulation The S1 and S2 regions exhibit a significant BOLD signal response variation that began and ended with pain stimulation, with the heightened BOLD response sustained through the entire pain period. Participants in the StimPVD and StimCON group had a similar pattern of variation during this time, although control participants showed greater variation in response to pain onset than women with PVD. There were no significant variations in the BOLD response patterns of S1 and S2 for the no stimulation conditions in either group. Within the amygdala, both StimCON and StimPVD participants had significant BOLD signal response variations at the time that pain begins. Only StimCON participants had variation patterns in the amygdala that scaled with pain at the time that the pain period began. Variations found in the pain stimulation conditions for the amygdala were also not seen in the no stimulation conditions. Within the PPC, the StimCON group was the only group that also exhibited significant variations when the pain began, as well as scaling with pain and temperature. During the no stimulation condition, many regions showed no significant variation across the time course for both the PVD and control group, including the, PFC, amygdala, S1, and S2. The PFC was the only region that showed no significant variations across both groups and conditions.

Results related to group and condition. Within the ACC, both NoStimCON and NoStimPVD participants displayed significant variations when they were told not to expect pain, but this similarity ends when the pain stimulation begins. NoStimPVD participants did not show the significant decline in BOLD response near the onset of the pain stimulation like the NoStimCON group, and they did not show a significant increase like the StimPVD and StimCON group. The StimCON group exhibited significant variations in both the thalamus and the ACC when they were told to expect pain, and when the pain began and ended. StimPVD participants also showed significant variations in these regions, but only when they were told to expect pain and when the pain began. BOLD responses in the thalamus and ACC for women with PVD were greater than control participants when they were told to expect pain. Also, StimPVD participants had variations that scaled with temperature when they were told to expect pain. Both NoStimPVD and NoStimCON groups had significant variations around the time they were told not to expect pain; however, only the control group also showed significant variations when the pain stimulation period began.

The INS had similar patterns of BOLD response in StimCON and StimPVD participants at the beginning of the pain period. However, only the StimCON participants had variations that scaled with temperature around the time that pain stimulation began and displayed significant variations when pain stimulation ended. Women with PVD in both conditions exhibited significant BOLD signal variations in the INS when they were told what condition to expect. This consistency between conditions was not found in the control group—only the NoStimCON participants had significant variation patterns during this period, in addition to having variations that scaled with temperature.

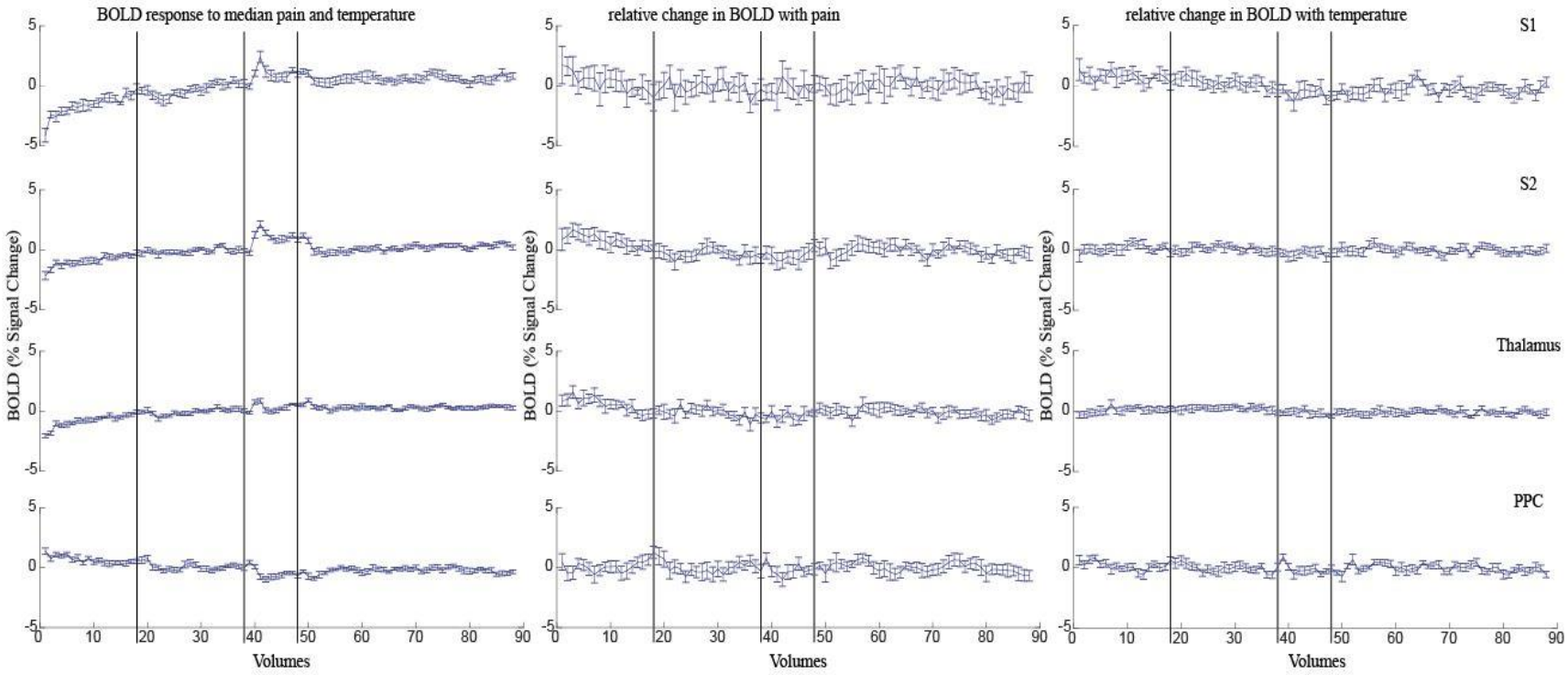

Figure 19. Brain StimCON Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

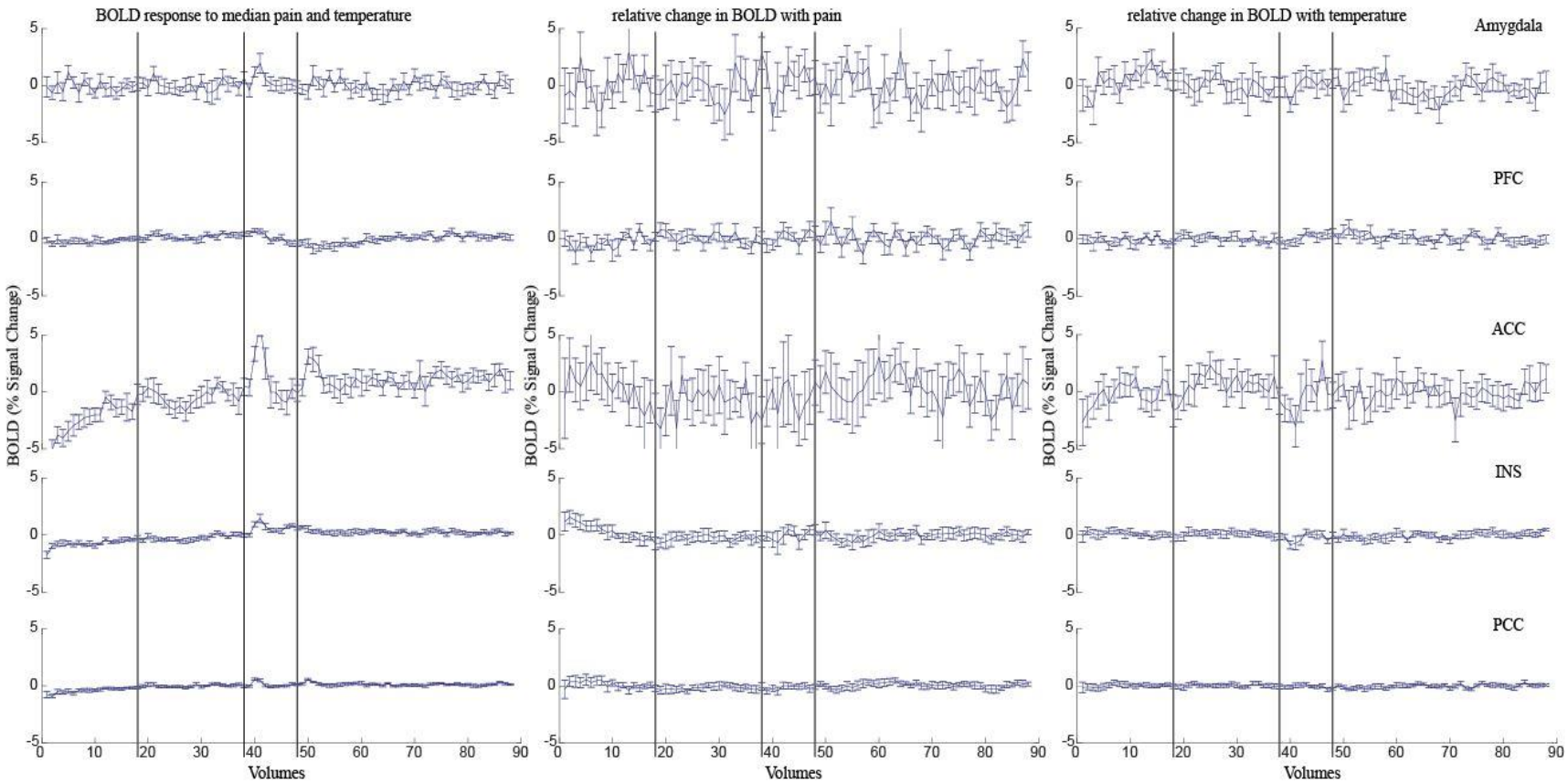

Figure 19. Brain StimCON Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

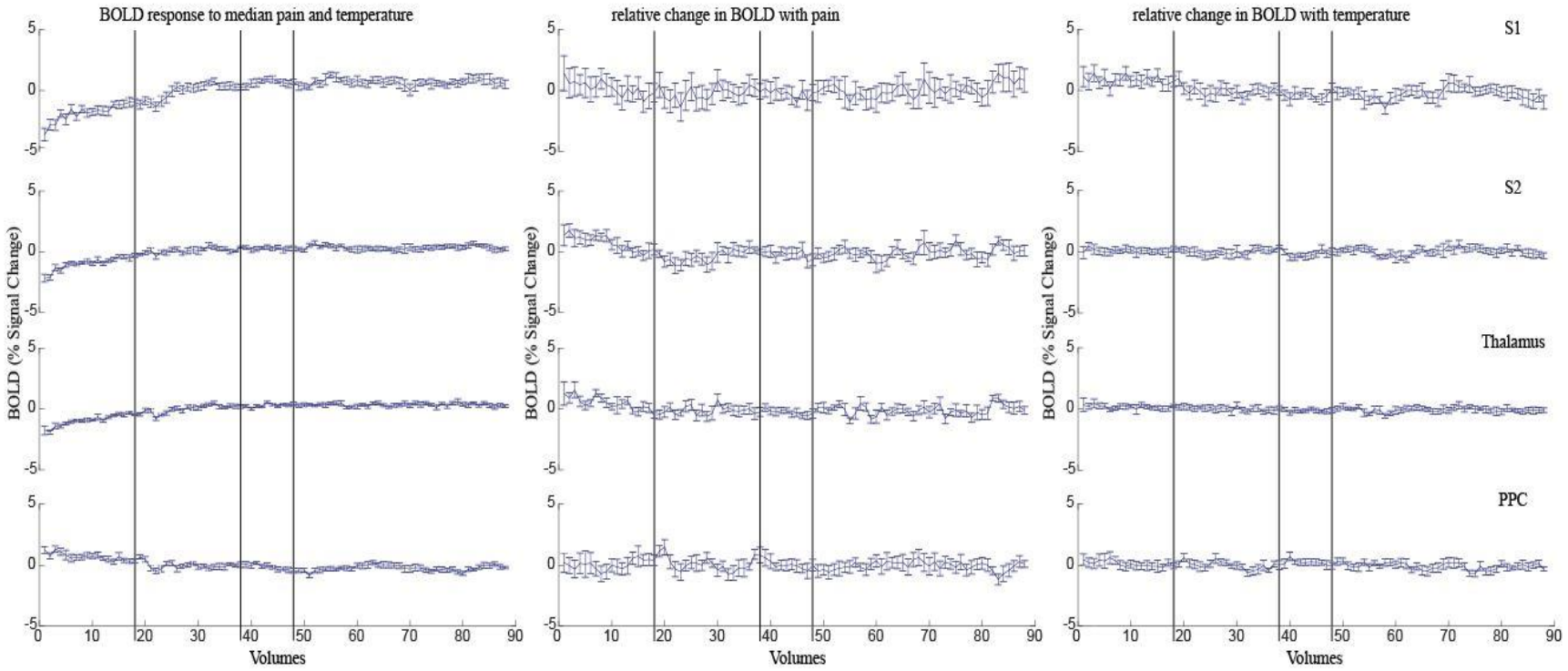

Figure 20. Brain NoStimCON Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

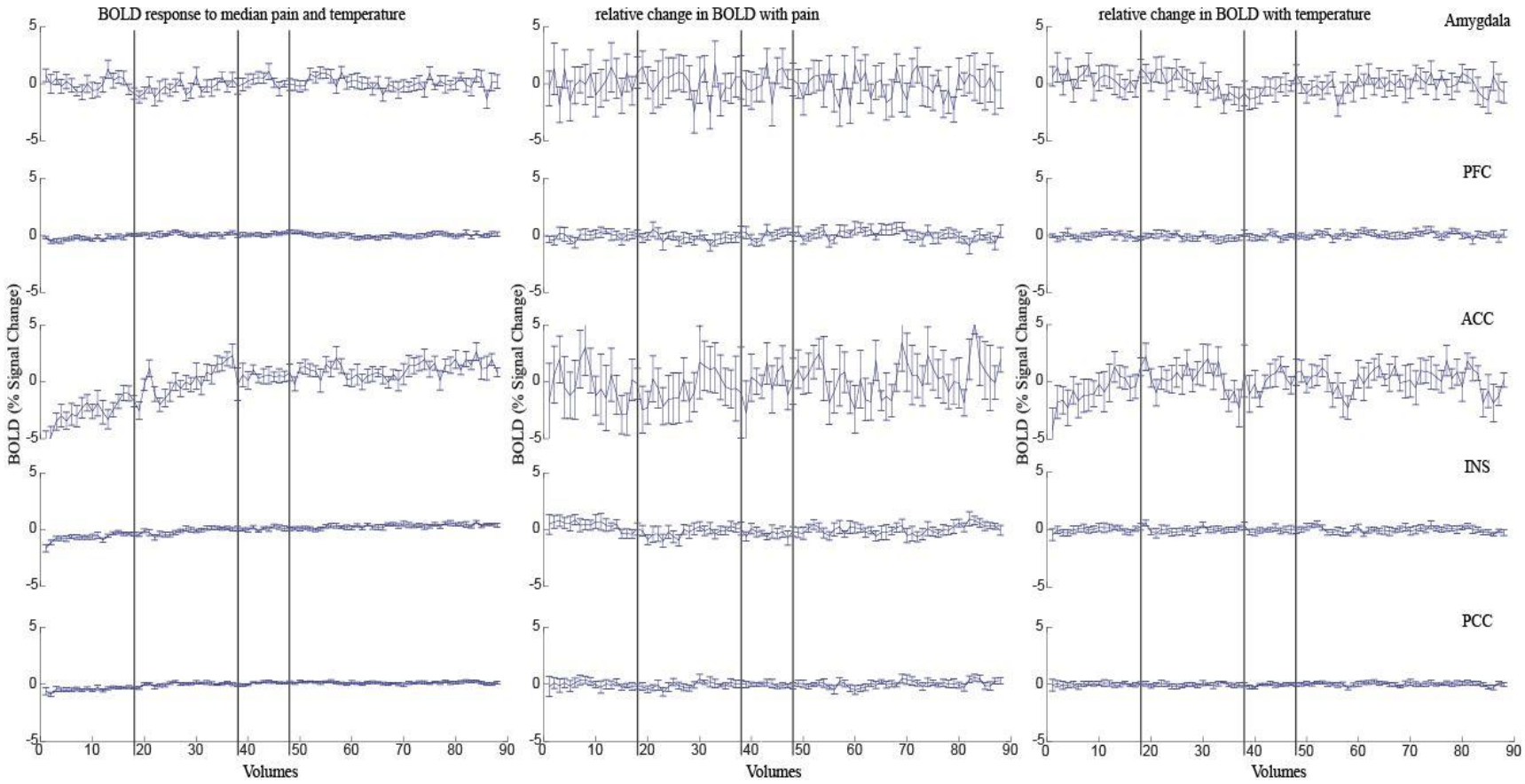

Figure 20. Brain NoStimCON Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

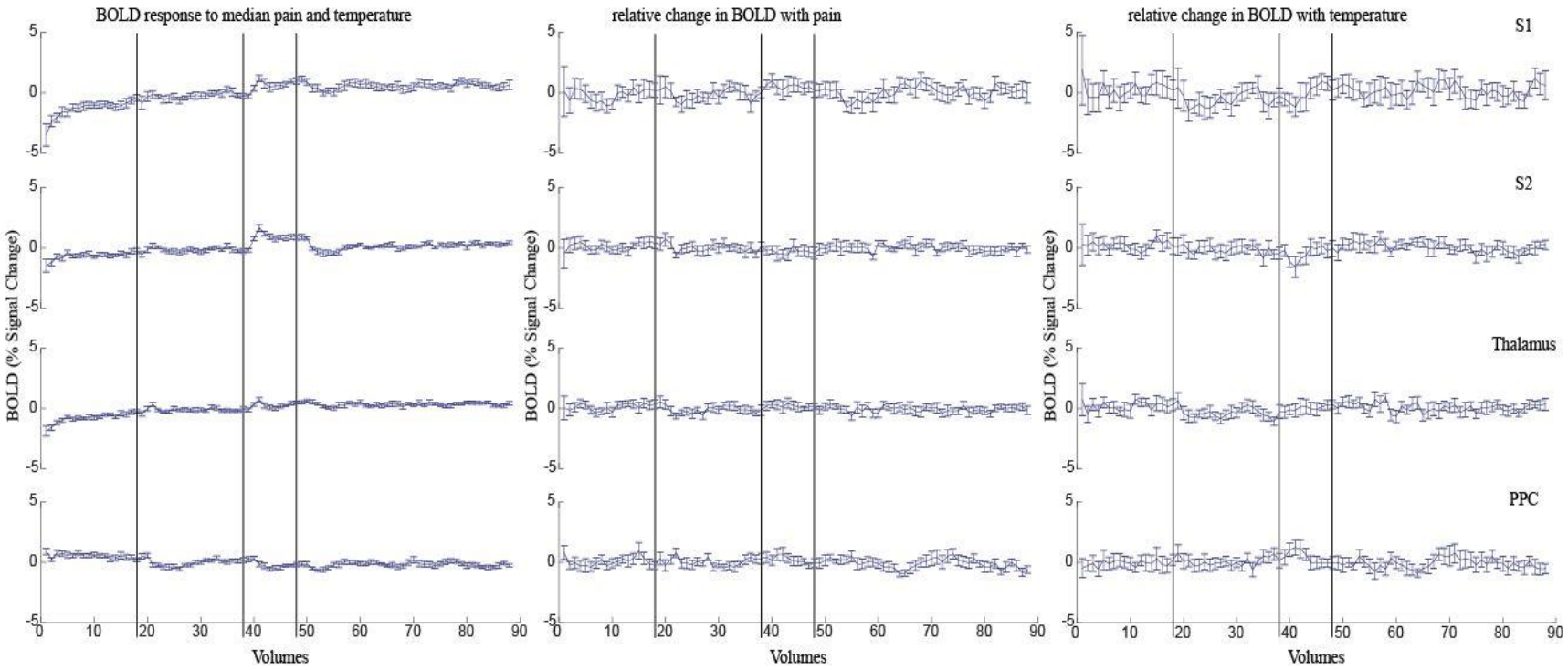

Figure 21. Brain StimPVD Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

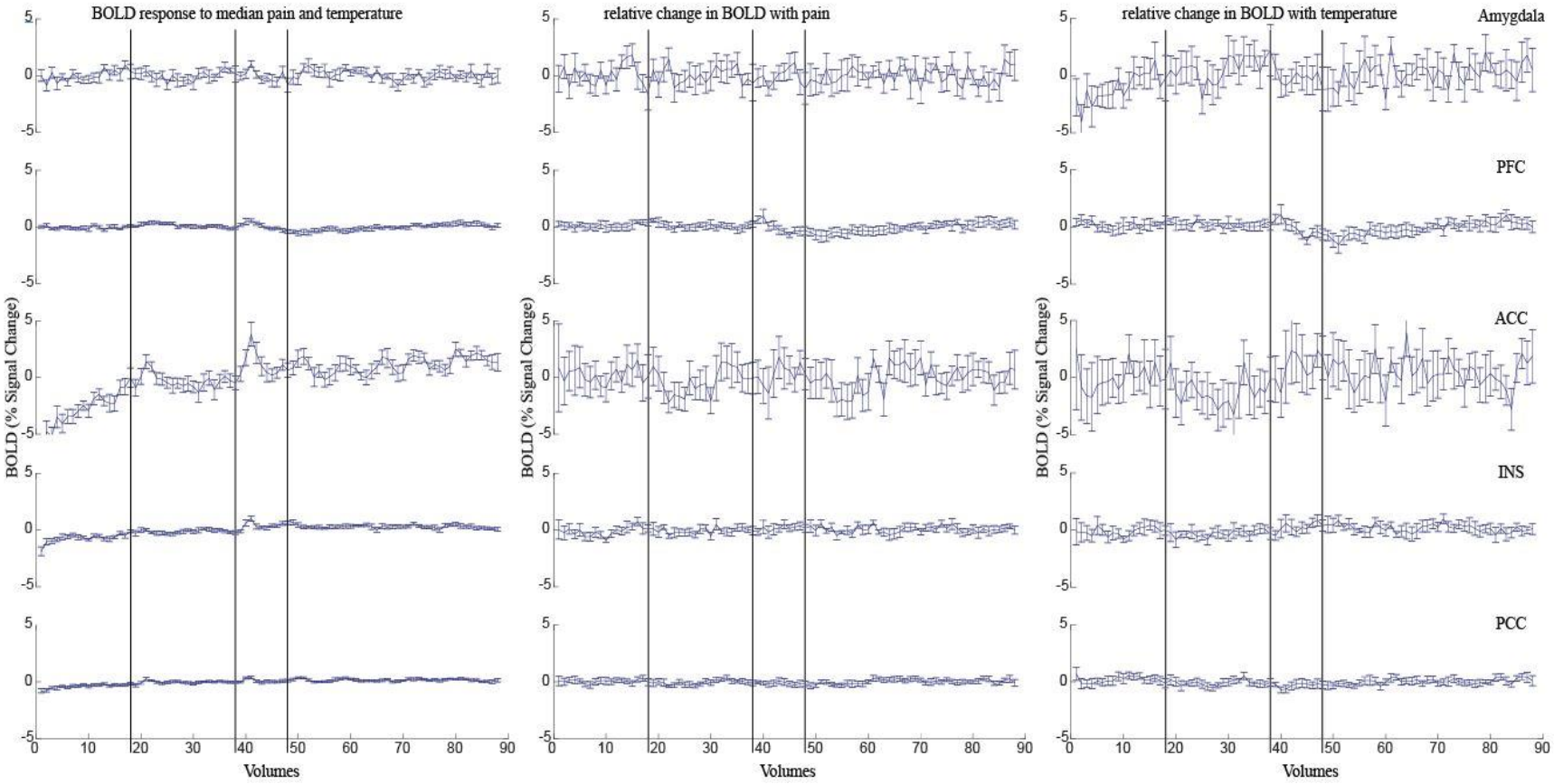

Figure 21. Brain StimPVD Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

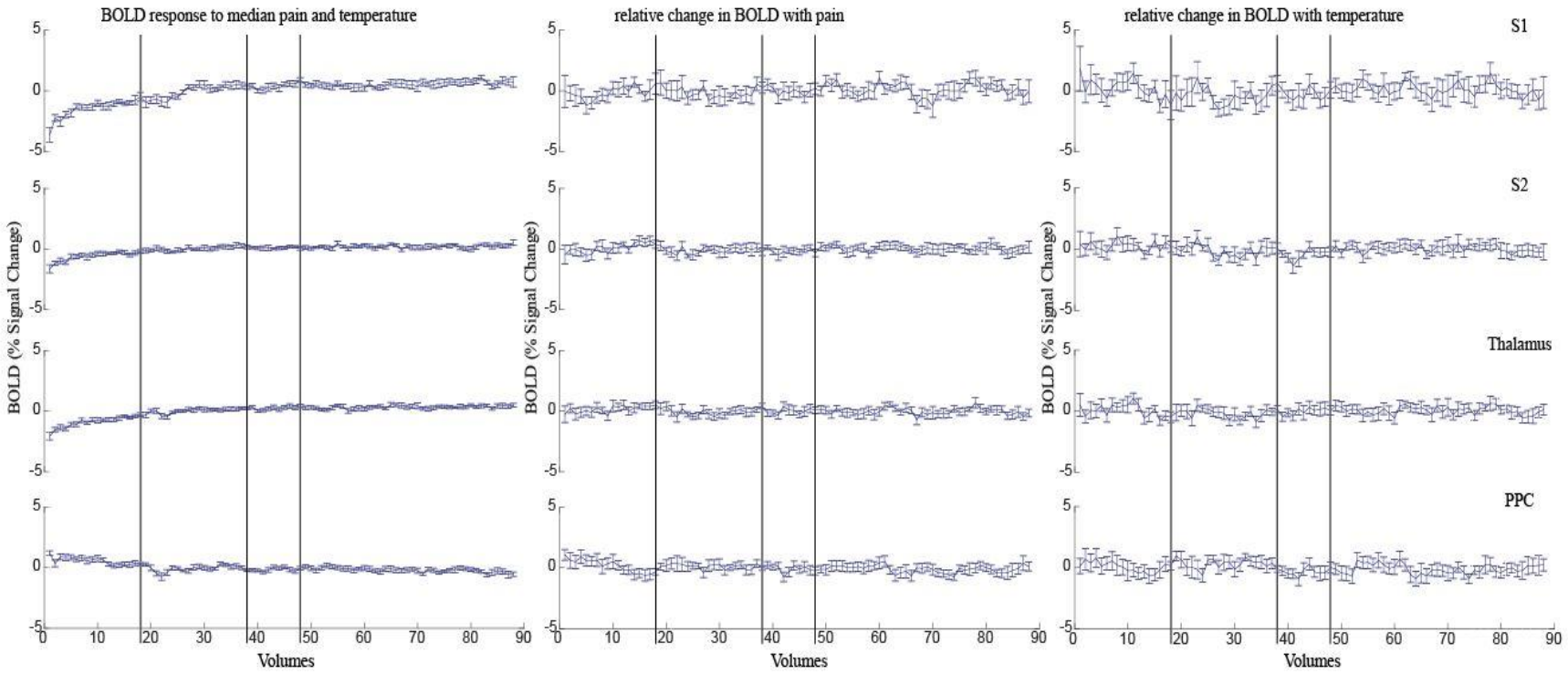

*Figure 22***.** Brain NoStimPVD Bayesian Regression. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

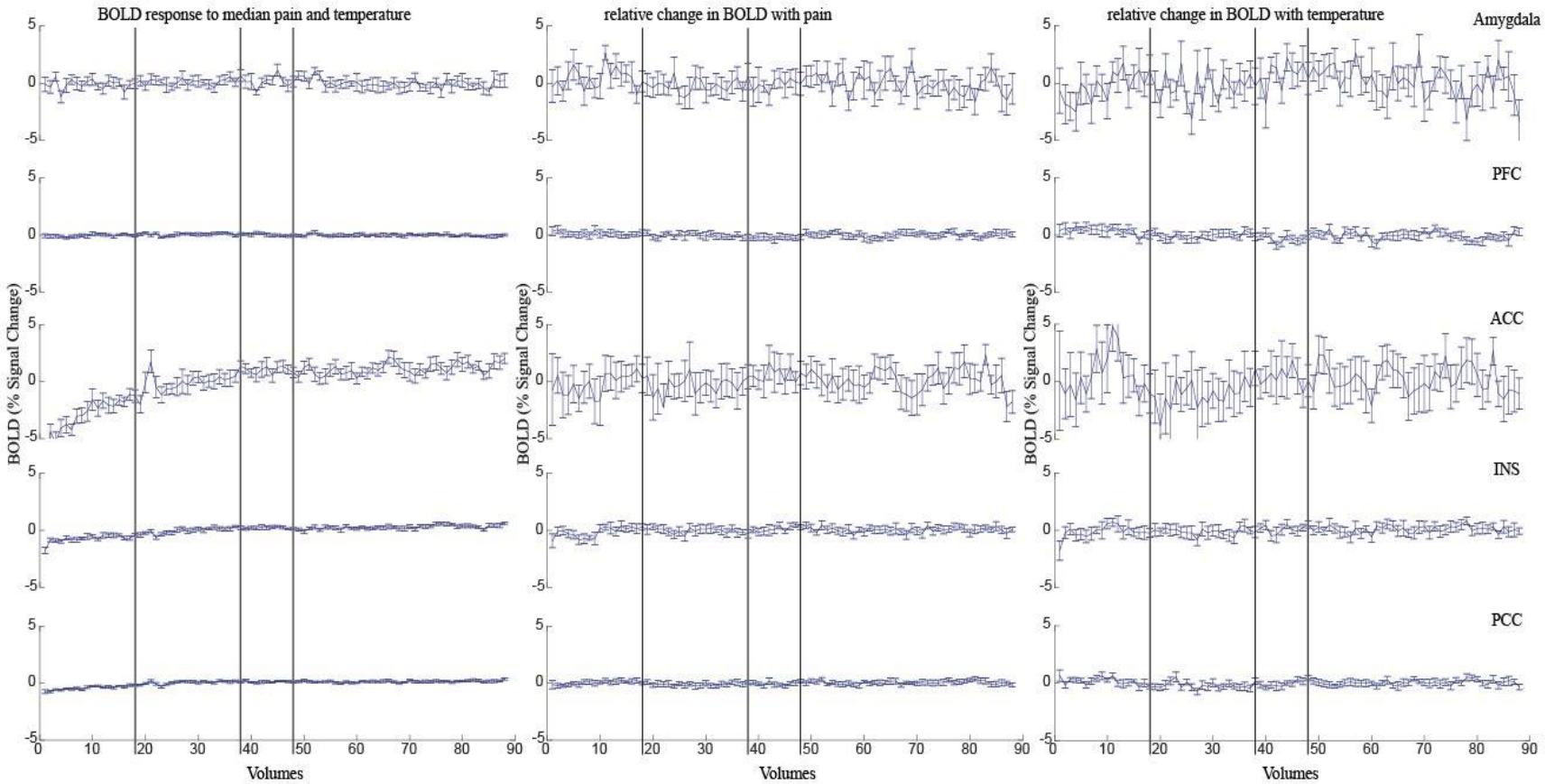

Figure 22. Brain NoStimPVD Bayesian Regression Continued. Displayed are the median BOLD response patterns (left), the variation with pain ratings (middle), and the variation with stimulation temperature (right).

Chapter 4

Discussion

This study is the first to examine spinal cord/brainstem connectivity and BOLD response in women with PVD; this study also investigated brain responses to a pain stimulation condition and a no stimulation condition for participants in the PVD and control groups. The aim of this study was to investigate if women with PVD exhibited any modification of descending modulatory mechanisms compared to healthy control participants. Results indicated that women with PVD displayed alterations in regions of the spinal cord/brainstem and brain known to play a role in pain perception.

Alterations in Connectivity

Based on the pattern of *t*-test results, one can observe that only women with PVD displayed any significant connectivity when no stimulation was applied, and they always exhibited weaker connectivity among the associated regions. Among those regions that were significantly different between StimPVD and StimCON women, women with PVD had many negative connections between regions that demonstrated a positive relationship in control participants. Control participants had more significant differences between the conditions and displayed a greater number of consistent connections than women with PVD. SEM analyses of the brain revealed that the consistent significant networks within each group were relatively similar between women with PVD and control participants. However, there were significant differences in the strength of connectivity between groups. Alterations in connectivity, and in particular a reduction in connectivity of the descending pain modulatory network, has been found in studies of other chronic pain disorders and could play a role in the clinical outcomes of PVD.

Spinal Cord/Brainstem SEM. Participants in the control group had more consistent connectivity across all periods of stimulation (i.e., before stimulation, during stimulation, and after stimulation) than women with PVD, particularly beginning in the hypothalamus and PAG. Similar to the PAG, the hypothalamus has been found to play an integral role in pain processing, and stimulation of the hypothalamus can inhibit the response of neurons in the spinal cord to painful stimuli (Millan, 2002). The hypothalamus is interconnected with the PAG, including the medial preoptic nucleus of the hypothalamus, which projects to the PAG. The antinociceptive relays known to exist between the hypothalamus and PAG—and many other regions including the NTS, NRM, and corticolimbic regions important to affective pain processing (Millan, 2002)—are illustrated by the significant connectivity found in the control participants of this study. More consistent connectivity in control participants amongst regions of descending pain modulation might indicate greater pain processing function in healthy participants compared to women with PVD, who exhibited patterns consistent with alterations of pain regulation.

When examining group level analyses, only women with PVD displayed any significant connectivity in the spinal cord/brainstem cord during the no stimulation condition, both before and during the stimulation period. Greater consistent connectivity during the no stimulation condition could suggest that women with PVD have alterations in pain processing even in the absence of provoked pain. Previous research used a resting state imaging paradigm to investigate groups differences in intrinsic connectivity in networks that are related to the dysregulation of pain processing, regions engaged at rest, and hypervigilance to pain that is seen in women with PVD (Gupta et al., 2015). Women with PVD had intrinsic connectivity in networks relevant to sensorimotor function and salience in the absence of any stimulation or paradigm change. However, the current study did not explicitly measure fMRI response during resting state. It only

included a no stimulation condition which cannot be equated to resting state; for instance, significant connectivity between these regions during the no stimulation condition could be in response to the knowledge that they will not be receiving pain stimulation. Despite the nonequivalence between a true resting state and the no stimulation condition, an understanding that women with PVD exhibit changes in networks integral to pain perception while in their baseline state could further our understanding of their group responses when no pain stimulation is present. However, a recent study has shown that there are BOLD signal variations during the periods that precede and follow a pain stimulus, and it is possible that these responses are not related to pain processing directly, but to cognitive, limbic, or autonomic functions (Stroman, Ioachim, Powers, Staud, & Pukall, 2018).

Neither of the connections that were significant in the SEM for NoStim conditions for each group were significantly different. In other words, although these connections were more consistent in the PVD group than in the control group, there were no meaningful differences in these connections between the groups or conditions.

During both the Stim and NoStim conditions, women with PVD had significant connectivity beginning in the C6 that was not seen in control participants. Input from the C6 to the NRM and NGC could be evidence of augmented ascending connectivity from the spinal cord to supraspinal structures of pain modulation in women with PVD (Millan et al., 2002). Three of the four significant connections for women with PVD were giving input to the NRM, a region that is not included in any significant connections of the control participants. The NRM is a nucleus of the RVM, which is important for both the inhibition and facilitation of nociceptive stimuli, and acts as a relay in descending pain facilitation (Ossipov, Morimura, & Porreca, 2014, p. 4). The RVM is a region that has long been examined for its role in moderating nociceptive

processes, including both analgesia and the development of chronic pain (Millan, 2002; Staud, 2013). As Staud (2013) explains, pain modulation is reliant on the responses of cells found in the RVM responsible for descending facilitation (i.e., ON cells) and inhibition (i.e., OFF cells) of pain. Hyperalgesia is the primary symptomology of chronic pain conditions, and it is believed that in animal models of hyperalgesia, there is an increase in activity of these ON cells (Staud, 2013; Bederson, Fields, & Barbaro, 1990). Alterations of NRM connectivity and activity in women with PVD in comparison to women without PVD could be evidence of dysfunction in a region related to pain chronicity.

Spinal Cord/Brainstem SEM Significant Differences. When comparing control and PVD participants receiving stimulation, the β-values for control participants are consistently significantly greater than PVD participants, and, in fact, PVD participants have reverse signs in seven of the 17 connected regions identified as being significantly different between these groups across the entire study paradigm. There is limited previous research examining brainstem connectivity in clinical pain populations. However, a study examining connectivity in women with fibromyalgia found that control participants displayed significantly higher connectivity from the ACC to multiple brain regions including the brainstem and had significantly higher connectivity from the thalamus to the orbitofrontal cortex (Jenson et al., 2012). Patients with fibromyalgia never exhibited greater connectivity than controls. Also, a study examining fMRI response within the brainstem of women with fibromyalgia found greater responses in the RVM, C6, NTS, LC, and the PAG (Bosma et al., 2016). Reverse direction of the correlation and lesser strength in the connectivity between these regions related to pain processing could be further evidence of alterations in descending pain processing in women with PVD. This pattern is not found during the no stimulation condition, suggesting that these differences are related to pain

processing in response to moderately noxious stimulation. Our results are consistent with the limited literature examining changes in brainstem regions of other chronic pain conditions.

When comparing those regions of connectivity that accounted for the greatest variance across both groups and conditions, connections incorporating the PAG and hypothalamus once again accounted for a large portion of significantly different results. A previous study of patients with spinal cord injuries (SCI) found that connectivity between regions in the brainstem was altered in participants with SCI in comparison to healthy control participants (Stroman, Khan, Bosma, Cotoi, Leung, Cadotte, & Fehlings, 2016). Control participants had considerably more connections that were correlated with pain than participants with SCI, who only had significantly correlated ratings with hypothalamus to NRM connectivity. Stroman et al. found that despite the wide variation in the results for SCI participants, both connectivity with the hypothalamus and descending modulation through the PAG-RVM-cord pathway (i.e., neural pathway important for descending antinociception) varied in relation to pain ratings and injury severity. Alterations of hypothalamus and PAG connectivity in women with PVD could indicate dysfunction of descending pain regulatory processes that have been found in brainstem studies of other chronic pain conditions (Stroman et al., 2016; Bosma et al, 2016; Burgmer et al., 2011). As further evidence of the role of PAG and hypothalamus connectivity in PVD, there was a significant correlation between pain ratings and connectivity strengths of PAG→HYP connectivity for the StimPVD group during pain stimulation. This correlation was not significantly associated with pain in control participants; however, NTS→PBN connectivity strength was correlated with pain ratings. These correlations suggest that perhaps connectivity between these separate network components play an essential role in integrating perceived pain—as defined by pain ratings in response to heat—into processing of pain information for each group.

Connectivity from the PAG to NTS was found to be significantly different and have reverse signs between the two groups in the no stimulation condition, both during and after stimulation. The NTS is known to be reciprocally interlinked with the PAG. It is a region that integrates autonomic and sensory systems, and it is from this location that descending inhibitory and facilitatory pain pathways originate (Millan et al., 2001). The NTS gives rise to two conflicting mechanisms of pain regulation; however, descending modulation of pain consists of a balance of both inhibition and facilitation that can result in the development of chronic pain if either system is dysregulated (Ossipov et al., 2014). NoStimPVD participants had greater connectivity strength between these regions than the NoStimCON group during both periods of the paradigm. This heightened connectivity in combination with the inverse direction of the relationship between the PAG and NTS indicates an intrinsic difference in regions that modulate pain processing in the dorsal horn of the spinal cord between women with and without PVD even in the absence of a pain stimulus.

When considering women with PVD between conditions, only connectivity from the C6 right dorsal to the thalamus was found to be significantly different during stimulation. This finding is in stark contrast to the control group who had 13 significantly different connections across the study paradigm. Women with PVD appeared to display fewer significant differences between stimulation and no stimulation conditions. If women with PVD have similar connectivity when they are and are not exposed to pain, then perhaps this pattern suggests that women with PVD consistently exhibit altered pain processing on a neurophysiological level, in contrast to healthy people without chronic pain who appear to vary between the two states. The only significant difference between the conditions for women with PVD included connectivity from the C6 right dorsal to the thalamus, which is not surprising. The spinothalamic tract, a

pathway leading from the periphery to the thalamus, projects sensory information to the thalamus to be relayed to regions of sensory integration, such as the S1, S2, IC, and ACC (Apkarian et al., 2005). The correlation between these two regions was significantly lower for women with PVD than control women. These results are consistent with patterns found in the chronic pain literature, as indicated by the meta-analysis conducted by Apkarian et al. (2005) who found that brain areas that are accessed through the spinothalamic tract have less activation in chronic pain populations. This diminished connectivity could be an indication of diminished ascending connectivity between these two regions in women with PVD that are essential for the integration of pain sensory information, implying distinct differences in the networks of spinal cord/brainstem regions related to pain perception in women with, versus those without, PVD. Without the proper integration of pain sensory information, one cannot expect the appropriate recruitment of pain modulatory mechanisms.

Connections originating from the C6 segment of the spinal cord also seem to hold some importance for pain processing in women with PVD. These connections were found to be significantly different when comparing stimulation and no stimulation conditions for women with PVD, as well as when comparing the stimulation conditions between groups. In both cases of significantly different connectivity (C6 $\rightarrow$ Thalamus, C6 $\rightarrow$ DRt), StimPVD participants had significantly lower β-values, even in comparison to the NoStimPVD condition. These results suggest that C6 connectivity may be particularly crucial for alterations in pain processing of PVD, because there were no significant differences for the comparison of control conditions to one another or the no stimulation conditions between groups for these regions, indicating the difference occurs in the presence of pain stimulation. These results seem consistent with the aforementioned meta-analysis—it is possible women with PVD have diminished ascending

connectivity deriving from the spinal cord, explicitly when reacting to a painful stimulus (Apkarian et al., 2005).

Brain SEM. SEM analyses of the brain on the group level revealed a relatively similar trend in the most consistent network components for each group. With a few exceptions, both women without chronic pain and women with PVD seem to recruit similar regions important to pain perception. Previous fMRI research of women with PVD and other chronic pain conditions have consistently found few differences in the regions recruited during pain stimulation paradigms. Pukall et al. (2005) found similar areas of activation in brain regions responding to an equivalent heat stimulus for both control and PVD participants. The primary differences were found in the degrees of activation within these regions, instead of the regions that were involved in pain processing, and this pattern is consistent with the results of this study. As discussed further, we found differences in BOLD signal variations of women with PVD, but differences in regions of connectivity were limited in comparison to the spinal cord and brainstem.

The thalamus is considered a relay station for all somatosensory information to regions of sensory perception (Yen & Lu, 2013). These regions include the S2, a region of somatosensory processing including nociceptive stimuli, that has been shown to be correlated with hypersensitivity in women with fibromyalgia and PVD (Pukall et al., 2005; Gracely et al., 2005). There was consistent Thalamus $\rightarrow$ S2 connectivity in the NoStimCON group that is not seen in the StimCON participants. The opposite is true for women with PVD. Women with PVD have previously displayed greater activation of the S2 in response to both non-painful and painful stimuli applied to the vulvar vestibule (Pukall et al., 2005). More consistent connectivity within each group during opposing condition types could indicate alterations of pain processing mechanisms specific to the application of a painful stimulus.

The amygdala, PFC, and PPC are all regions of emotion regulation, salience, and sensory integration (Bernard, Bester, & Besson, 1996). Regions responsible for salience monitor homeostatic changes in the body (Borsook et al., 2013). The control group in both conditions had more consistent connectivity between Amygdala → PFC and S2 → PPC across the whole study paradigm than women with PVD. Previous research has found that women with fibromyalgia exhibit less connectivity from the thalamus to the PFC than women without chronic pain (Jensen et al., 2012). In addition, the PPC is important for the integration of somatosensory information and spatial integration of painful stimuli (Oshiro, Quevedo, McHaffie, Kraft, & Coghill, 2009). Many of these aforementioned regions are part of connectivity network components that are found to be significantly different between women with PVD and control participants.

Brain SEM Significant Differences. Despite there being limited differences between groups in the group level SEM, there were significant differences in the β-values of regions in the connectivity network components that accounted for the greatest variance. When comparing the groups and conditions to examine significant differences in the connectivity of network components, control participants have notably fewer significantly different connections than women with PVD. Previous examinations of brain connectivity in women with PVD and fibromyalgia have found greater connectivity in regions in the salience network (Gupta et al., 2015; Jenson et al., 2012). Women with PVD have also displayed differences in activation of regions related to emotional arousal and response to pain (Pukall et al., 2005; Pazmany et al., 2017). This difference in activity is expected as women with PVD have more mental health issues and higher rates of pain catastrophizing than women without chronic pain (Pukall et al., 2002; Pukall et al., 2016).

Before painful stimulation, there were significant correlations between pain ratings and connectivity strengths but in different connectivity regions. Control participants had a significant correlation between pain and S1→S2 connectivity. This correlation with regions of sensorimotor processing is in contrast to regions of salience and emotional integration found to be correlated with pain in PVD participants (ACC→Amygdala, INS→Amygdala). It is possible that because women with PVD exhibit more emotion dysregulation, that they exhibit more differences in regions related to pain cognitions. Therefore, they may have more significant differences in regions of salience and emotional integration than control participants between conditions of stimulation.

When comparing the significantly different connections between conditions for women with PVD, a majority of differences involved the thalamus, S1, and S2 as source or target regions. The thalamus, S1, S2, and PPC were also regions that made up a majority of the significantly different connections between the StimCON and StimPVD groups. All four of these regions have been previously shown to play an important role in the processing of tactile sensory information (Davis, Kwan, Crawley, & Mikulis, 1998; Olausson et al., 2001). Previous examination of connectivity involving the thalamus in women with fibromyalgia has shown significantly less connectivity from the thalamus to the orbitofrontal cortex and no regions in which fibromyalgia patients had higher thalamic connectivity than control participants (Jensen et al., 2012). Patients with fibromyalgia also show significantly greater activation of the PPC in anticipation of pressure pain to the thumb as compared with non-affected participants (Burgmer et al., 2011). In this study, women with PVD displayed significantly different connectivity from healthy control participants in these regions of somatosensory information processing. This

difference in connectivity is consistent with previous studies of chronic pain populations that have also found alterations in the connectivity and functional response of these regions.

Alterations in BOLD Response

There were many regions of the spinal cord/brainstem that had significantly different BOLD responses between participant groups. However, the direction of these differences varied by region, as the variations in BOLD response were greater in women with PVD than control participants for certain regions (e.g. C6 and PAG) but less for other regions (e.g. NRM and NTS). Frequently the BOLD responses between the two groups depicted an inverse pattern. In addition, women with PVD in multiple instances did not show differences between stimulation conditions that were seen in control participants, meaning the NoStimPVD and StimPVD BOLD responses were often more similar to each other than control participants in the spinal cord/brainstem.

Despite having similar regions connected in the brain between the group, there were significant alterations in the BOLD signal responses of women with PVD. In certain regions, the groups differed at what point of the study paradigm they exhibited significant variations (e.g. thalamus and ACC). Control participants had greater BOLD signal variations in individual regions (PPC and S1); however, similar to the spinal cord/brainstem data this difference varied by region and women with PVD did have greater bold responses in certain periods of the study paradigm (e.g. thalamus and ACC). These regions are all essential to the integration of sensory information and the function of pain regulatory systems. As previously stated, there is a continual balance of inhibition and facilitation within the descending regulatory mechanisms and an imbalance of either can lead to the development and maintenance of a chronic pain condition.

Spinal Cord/Brainstem Bayesian Regression. There was no significant difference in connectivity either originating from or targeting the NRM; however, we do see differences in the BOLD signal response variations between groups. During pain stimulation, the StimCON participants had significant variations that scaled with pain, in comparison to the NoStimCON group who did not have significant variations. This difference between the stimulation conditions was not found in the PVD population who showed no significant response during the pain period in either condition. These results are consistent with previous findings in the chronic pain literature. A study of women with fibromyalgia using a heat pain stimulus found greater activation in the RVM in control participants than in patients with fibromyalgia (Bosma et al., 2016). As previously mentioned, the RVM has an integral role in descending pain modulation, and the dysregulation of regions related to pain processing could impact the development of chronic pain.

There were also significant BOLD signal response variations in the C6 during pain stimulation that scaled with pain and temperature in women with PVD that are not seen in the control group. Of the limited studies examining spinal cord activation in clinical populations, a study of participants with multiple sclerosis (MS) found that patients with MS had more frequent activation in the C5 and C6 portions of the cervical cord (Agosta, Valsasina, Caputo, Stroman, & Filippi, 2008). The impact of pain and temperature on the BOLD signal response for PVD participants would suggest that the spinal cord is important for integrating the effects of the intensity and perceived pain of the heat stimulus in women with PVD.

In the PAG before pain stimulation, women in the StimPVD group had similar patterns of BOLD signal variation to both of the no stimulation conditions, but the StimCON group had an inverse pattern to all three. The StimCON group had a declining BOLD signal response

following the indicator that they would feel pain in comparison to the other groups. These results are consistent with previous studies of activation in women with fibromyalgia who had greater activation in the PAG than control participants in response to anticipation of pressure pain applied to the thumb (Burgmer et al., 2001). During the heat stimulus period, the PAG and hypothalamus did not show significant signal variations; however, the PAG scaled with pain and temperature for the StimPVD, StimCON, and NoStimPVD groups. StimCON participants also displayed variations that scaled with pain in the hypothalamus. The BOLD signal response during the stimulation period between StimCON and StimPVD participants was similar—this similarity is in contrast to research of women with fibromyalgia. Bosma et al. (2016) found that control participants had significantly greater activation in the PAG than women with fibromyalgia in response to a heat stimulus paradigm. It should be noted that neither the PAG nor the hypothalamus appeared to be influenced by pain or temperature in the NoStimCON group, raising the question of whether there is dysregulation in women with PVD so that their PAG BOLD signal variations in the no stimulation condition are more similar to those in the pain condition. There was also an inverse pattern of variation in the hypothalamus between the no stimulation control participants and women with PVD at the time they were told whether or not to expect pain. Women with PVD exhibited altered pain processing from control women who had much greater connectivity from these pain modulatory regions.

The NoStimCON group during the pain stimulation period had significant variations in their NTS BOLD signal responses that scaled with pain ratings, which was not found in the NoStimPVD group. In addition, the patterns of variations between these two groups during this period were inverse. However, the patterns of variations between women with PVD and control participants were similar in the time after pain stopped. Similar results have been found in

studies using a temporal summation of second pain control (TSSP-C) paradigm in fibromyalgia participants. TSSP-C is performed by applying a repeated heat stimulus with enough duration between each contact that there is no summation of pain over time in healthy control participants (Bosma et al., 2016). They found greater fMRI responses in control participants than participants with fibromyalgia in response to the TSSP-C condition.

Brain Bayesian Regression. StimPVD and StimCON participants show similar patterns of BOLD signal variation in the S1 and S2. The BOLD response began and ended with pain stimulation, with the heightened BOLD response sustaining through the entire pain period, but control participants displayed greater S1 variation in response to the onset of pain than women with PVD. These BOLD response patterns were not seen in the no stimulation condition. Previous fMRI examinations of women with PVD have found no significant activation in the S1 for either women with PVD or control participants (Pukall et al., 2005). It is possible that these contradictory results are due to differences in the pain stimulation paradigms. In the Pukall et al. (2005) study, control participants were given the same stimulus as the titrated stimulation intensity as their matched counterpart, whereas in the current study, women in both groups were given a stimulus based on individually titrated pain response. It is possible that the intensity of the stimulus for control participants was relatively higher in this study than in the previous fMRI study of women with PVD. The thresholds used in the previous study were based on the thresholds of women with PVD, therefore leading to lower stimulation of the S1 and not revealing a significant difference between groups.

The amygdala also displayed similar patterns of variation in both StimCON and StimPVD participants when pain began. Furthermore, StimCON participants had variations that scaled with pain at the time that the pain stimulation began. Similar to the S1 and S2 regions,

these variations were not seen in the no stimulation condition. The amygdala is a region known to be related to emotional-arousal regulation in pain processing. Control and PVD participants appear to have a similar response in the amygdala when pain begins, perhaps their emotional response to the sudden pain stimulation. This similarity is contradictory to studies of women with PVD; for instance, Pazmany et al. (2017) found a greater response in the amygdala of women with Genito-Pelvic Pain/Penetration Disorder (GPPPD)—a diagnostic label which includes vulvodynia and its subtypes. However, similar to their results, we also did not observe any differences in region activation when participants were told to expect heat stimulation. Participants with GPPPD did not display any differences in activation during anticipation of pain in comparison to healthy control participants (Pazmany et al., 2017).

Within the PPC, participants in the StimCON group had significant variations when the pain stimulation began and scaled with pain and temperature, which is not seen in StimPVD participants. These results conflict with previous studies of parietal cortex activation in women with PVD as well as fibromyalgia patients. In the inferior parietal lobule, women with PVD had activation that was associated with clinical pain outcomes (Hampson et al., 2013). Patients with fibromyalgia have been found to display greater activation in the PPC in anticipation of pressure applied to the thumb (Burgmer et al., 2011). Both of these studies used individually titrated pain intensity for each participant; however, neither used the same paradigm of repeated application of a moderately painful stimulus that was conducted in this study. There is no conclusive explanation for this contradiction with literature, but there is limited research examining fMRI response and connectivity of the PPC in women with PVD. Further research examining this region of sensory information could reveal a more consistent pattern of neuropathological pain dysfunction in a disorder of provoked pain.

The thalamus and ACC showed significant variations in the StimCON group when they were told to expect pain, when pain began, and when pain ended. The StimPVD group also displayed significant variations in these regions, but only when they were told to expect pain and when pain began. In addition, their BOLD responses when they were told to expect pain were greater than control participants and their variations scaled with temperature. These results are relatively consistent with previous research of functional modifications in women with PVD in these regions. Women with PVD have displayed greater activation of the ACC and the thalamus during pain induction in two separate studies (Pazmany et al., 2017; Hampson et al., 2013). However, in the study examining activation during a period of anticipation of pain, women with PVD did not have significantly greater activation in these regions (Pazmany et al., 2017). This null result could be considered contradictory to the outcomes of this study showing greater variation when they are told to expect pain stimulation. Nevertheless, these studies are the only two studies to examine fMRI response to anticipation of pain in women with PVD; therefore, future research can continue to illuminate the consistent regions of fMRI response for PVD during anticipation of pain.

StimCON and StimPVD participants also had similar patterns of INS BOLD signal response at the beginning of the pain period, but only the StimCON groups had variations that scaled with temperature when the pain stimulation began and significantly vary when pain stimulation ended. Women with PVD in both conditions displayed significant BOLD signal variations in the INS when they were told what condition to expect. This significant variation is not true of the control group, as only the NoStimCON participants had significant variation patterns during this period. The INS is a brain region that has been shown to be activated in response to pain (Coghill et al., 1994; Casey et al., 1996; Davis et al., 1998), and previous

investigations of INS activation in women with PVD has shown alterations in the activity in two separate studies. Women with PVD have displayed greater activation during painful thumb stimulation in the INS (Hampson et al., 2013). They have also displayed activation in the mid-posterior INS to non-painful pressure as well as bilateral activation in both the anterior and posterior INS (Pukall et al., 2005). The INS is an important location for transferring somatosensory information and pain processing (Derbyshire et al., 1997; Coghill et al., 1994; Casey et al., 1994). We interpret these results as further evidence of augmented pain processing in regions that commonly respond to painful stimulation in women with PVD.

QST Outcomes

The ratings reported by both PVD and control participants in response to the titrated heat stimulus applied to the hand were extremely similar. Although group differences were in the expected direction—in that women with PVD had lower moderate pain thresholds than control women—they did not reach statistical significance. This pattern of results differs from what is usually found in the literature. Typically, QST examinations of non-vulvar pain stimuli in women with PVD have demonstrated significant levels of hypersensitivity in multiple regions of the body in comparison to healthy control participants (e.g., Pukall et al., 2006; Bohm-Starke et al., 2001; Giesecke et al., 2004; Granot et al., 2002; Pukall et al., 2002). The lack of a significant difference in QST outcomes in the present study could be due to the specific methodology used. The heat stimulus targeted a moderate pain rating within a protocol that involved the use of a pre-established range of temperatures, which differs from typically employed thermal QST protocols in the vulvodynia literature that assess thresholds via a method of limits or variations thereof (e.g., Sutton, Pukall, & Chamberlain, 2009). Also, it is possible that this discrepancy is due to individual differences in the pain perception of PVD. It is recognized that there is much

variability in the experience of pain amongst healthy individuals, and perception of pain can vary widely even when injuries are similar (Staud, 2013). Individual differences in this population of women with PVD could impact their QST outcomes.

Limitations

When performing SEM, we chose regions of interest based on previous research of descending pain regulation for connectivity analyses. As a result, there could possibly be many regions relevant to pain processing that were not included in the network model. By including more regions to assure all possible anatomical structures are accounted for, we increase the number of comparisons performed and run the risk of including nonsignificant network connections that weaken the strength of β values for each network component. Also, when examining the β weights between two correlated regions, we interpret these results as direct connectivity between the two regions; however, it is possible that the connection is actually incorporating other regions that are not taken into account.

Four of the 16 women with PVD did not enter the sham MRI before their MRI session due to time constraints, although they were trained on the QST measures. The sham MRI is used to acquaint participants to the MRI environment and reduce any negative feelings such as anxiety associated with an unfamiliar and claustrophobic environment. When excluding these women in an SEM of the brainstem/spinal cord data, there did not appear to be any major differences in the SEM outcomes. However, this study did collect information on the self-report anxiety of participants during the MRI protocol, and taking into consideration the impact of psychosocial characteristics such anxiety and catastrophizing, we plan to further examine the role of these factors in future studies.

In addition to the limitation of SEM, PVD is a heterogenous patient group with a wide range of symptom severity, comorbidities, and psychosocial characteristics. It is likely that many of these factors could contribute to alterations in pain processing mechanisms, especially with the knowledge that emotional states play a significant role in pain perception (Villemure & Bushnell, 2002). Also, this study included women with both primary and secondary PVD. Previous comparisons of women with different subtypes of PVD have found differences in some characteristics of pain processing mechanisms, such as grey matter density (Sutton et al., 2015; Hampson et al., 2013). For this reason, there could be differences in the neural responses to pain stimulation among women with PVD based on subtype. This study did not have the power to examine differences between these groups; however, future examinations of spinal cord connectivity and activity should investigate differences between PVD subtypes.

Future Research

With an established protocol for the assessment of spinal cord activity and evidence of modifications in descending pain modulation mechanisms, future research should seek to examine commonalities among PVD and other commonly comorbid pain disorders, such as fibromyalgia, IBS, or interstitial cystitis. Previous research has examined brain activation among patients with PVD, fibromyalgia, and IBS; however, spinal cord imaging allows for a more complete understanding of descending pain modulation. Understanding the common neuropathological characteristics among these pain disorders that already share psychosocial and pain symptomology could further illuminate our understanding of the pathophysiology of PVD and its comorbid disorders.

Of the limited studies using fMRI techniques to examine women with PVD, one study has investigated and found alterations in the connectivity of sensorimotor, salience, and default

networks in women with PVD (Gupta et al., 2015). These three networks have been found to be altered in other chronic pain populations and appear to be related to the magnification of the pain state. Future research should also seek to examine the alterations in connectivity of the brainstem and spinal cord during task-free resting state fMRI studies. Research in this avenue could further clarify the results of this study and explain the possible impact of intrinsic differences in pain processing of women with PVD even when no stimulation is applied. The spontaneous regulation of descending pain modulatory regions in addition to that seen within the brain could contribute further evidence to the role of central mechanisms in the development of PVD.

Implications and Conclusions

This study is the first to examine both the spinal cord/brainstem and brain of women with PVD, providing a more complete understanding of central processing. The results demonstrate alterations in regions relevant to pain modulation in the brainstem and spinal cord, such as the hypothalamus, thalamus, NRM, PAG, NTS, and C6. Despite having similar connectivity between regions in the brain that are relevant to pain processing between women with and without PVD, this study contributes to a body of research that confirms alterations in the fMRI response of these brain regions in PVD not observed in control participants within a pain paradigm. These results suggest that PVD could be related to dysfunction in descending pain regulatory systems and provide further evidence that dysfunction of pain processing is not in fact a local problem to the vestibule, but instead highlights the role of central processes that contribute to pain perception in women with PVD. These results will continue to direct our attention towards alterations of central processes and further provide evidence that these differences in pain processing are not only present in response to noxious stimuli, but also in the absence of pain stimulation.

References

Agosta, F., Valsasina, P., Caputo, D., Stroman, P. W., & Filippi, M. (2008). Tactile-associated recruitment of the cervical cord is altered in patients with multiple sclerosis. *Neuroimage*, *39*(4), 1542-1548.

Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European journal of pain*, *9*(4), 463-484.

Apkarian, A. V., Sosa, Y., Sonty, S., Levy, R. M., Harden, R. N., Parrish, T. B., & Gitelman, D. R. (2004). Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *Journal of neuroscience*, *24*(46), 10410-10415.

Arnold, L. D., Bachmann, G. A., Kelly, S., Rosen, R., & Rhoads, G. G. (2006). Vulvodynia: characteristics and associations with co-morbidities and quality of life. *Obstetrics and gynecology*, *107*(3), 617.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). Beck depression inventory.

Bee, L., & Dickenson, A. (2009). Descending modulation of pain. In *Synaptic Plasticity in Pain* (pp. 307-335). Springer, New York, NY.

Bederson, J. B., Fields, H. L., & Barbaro, N. M. (1990). Hyperalgesia during naloxone-precipitated withdrawal from morphine is associated with increased on-cell activity in the rostral ventromedial medulla. *Somatosensory & motor research*, *7*(2), 185-203.

Bergeron, S., Binik, Y. M., Khalifé, S., Pagidas, K., & Glazer, H. I. (2001). Vulvar vestibulitis syndrome: reliability of diagnosis and evaluation of current diagnostic criteria. *Obstetrics & Gynecology*, *98*(1), 45-51.

Bernard, J. F., Bester, H., & Besson, J. M. (1996). Involvement of the spino-parabrachio-amygdaloid and-hypothalamic pathways in the autonomic and affective emotional aspects of pain. In *Progress in brain research* (Vol. 107, pp. 243-255). Elsevier.

Biswal, B., Zerrin Yetkin, F., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magnetic resonance in medicine, 34(4), 537-541.

Bogdanov, V. B., Viganò, A., Noirhomme, Q., Bogdanova, O. V., Guy, N., Laureys, S., ... & Schoenen, J. (2015). Cerebral responses and role of the prefrontal cortex in conditioned pain modulation: an fMRI study in healthy subjects. *Behavioural brain research*, *281*, 187-198.

Bohm-Starke, N., Hilliges, M., Brodda-Jansen, G., Rylander, E., & Torebjörk, E. (2001). Psychophysical evidence of nociceptor sensitization in vulvar vestibulitis syndrome. *Pain*, *94*(2), 177-183.

Bornstein J, Goldstein AT, Stockdale CT, Bergeron S, Pukall C, Zolnoun D, Coady D (2016). 2015 ISSVD, ISSWSH and IPPS Consensus terminology and classification of persistent vulvar pain and vulvodynia. *Obstetrics and Gynecology*, *127*, 745-751.

Bornstein, J., Goldschmid, N., & Sabo, E. (2004). Hyperinnervation and mast cell activation may be used as histopathologic diagnostic criteria for vulvar vestibulitis. *Gynecologic and Obstetric Investigation*, *58*(3), 171-178.

Borsook, D., Edwards, R., Elman, I., Becerra, L., & Levine, J. (2013). Pain and analgesia: the value of salience circuits. *Progress in neurobiology*, *104*, 93-105.

Borsook, D., Sava, S., & Becerra, L. (2010). The pain imaging revolution: advancing pain into the 21st century. *The Neuroscientist*, *16*(2), 171-185.

Bosma, R. L., & Stroman, P. W. (2014). Assessment of data acquisition parameters, and analysis techniques for noise reduction in spinal cord fMRI data. *Magnetic Resonance Imaging*, 32(5), 473-481.

Bosma, R. L., & Stroman, P. W. (2015). Spinal cord response to stepwise and block presentation of thermal stimuli: A functional MRI study. *Journal of Magnetic Resonance Imaging*, 41(5), 1318-1325.

Bosma, R. L., Ameli Mojarad, E., Leung, L., Pukall, C., Staud, R., & Stroman, P. W. (2015). Neural correlates of temporal summation of second pain in the human brainstem and spinal cord. *Human Brain Mapping*, 36(12), 5038-5050.

Bosma, R. L., Mojarad, E. A., Leung, L., Pukall, C., Staud, R., & Stroman, P. W. (2016). FMRI of spinal and supra-spinal correlates of temporal pain summation in fibromyalgia patients. *Human brain mapping*, *37*(4), 1349-1360.

Bouhassira, D., Moisset, X., Jouet, P., Duboc, H., Coffin, B., & Sabate, J. M. (2013). Changes in the modulation of spinal pain processing are related to severity in irritable bowel syndrome. *Neurogastroenterology & Motility*, 25(7), 623.

Burgmer, M., Petzke, F., Giesecke, T., Gaubitz, M., Heuft, G., & Pfleiderer, B. (2011). Cerebral activation and catastrophizing during pain anticipation in patients with fibromyalgia. *Psychosomatic medicine*, *73*(9), 751-759.

Cadotte, D. W., Bosma, R., Mikulis, D., Nugaeva, N., Smith, K., Pokrupa, R., ... & Fehlings, M. G. (2012). Plasticity of the injured human spinal cord: insights revealed by spinal cord functional MRI. *PLoS One*, 7(9), e45560.

Cahill, C. M., & Stroman, P. W. (2011). Mapping of neural activity produced by thermal pain in the healthy human spinal cord and brain stem: a functional magnetic resonance imaging study. *Magnetic Resonance Imaging*, *29*(3), 342-352.

Casey, K. L. (1996). Match and mismatch: identifying the neuronal determinants of pain. *Annals of Internal Medicine*, 124(11), 995-998.

Casey, K. L., Lorenz, J., & Minoshima, S. (2003). Insights into the pathophysiology of neuropathic pain through functional brain imaging. *Experimental neurology*, *184*, 80-88.

Casey, K. L., Minoshima, S., Berger, K. L., Koeppe, R. A., Morrow, T. J., & Frey, K. A. (1994). Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. *Journal of neurophysiology*, 71(2), 802-807.

Coghill, R. C., Gilron, I., & Iadarola, M. J. (2001). Hemispheric lateralization of somatosensory processing. *Journal of Neurophysiology*, 85(6), 2602-2612.

Coghill, R. C., Sang, C. N., Maisog, J. M., & Iadarola, M. J. (1999). Pain intensity processing within the human brain: a bilateral, distributed mechanism. *Journal of Neurophysiology*, 82(4), 1934–1943.

Coghill, R. C., Talbot, J. D., Evans, A. C., Meyer, E., Gjedde, A., Bushnell, M. C., & Duncan, G. H. (1994). Distributed processing of pain and vibration by the human brain. *Journal of Neuroscience*, 14(7), 4095-4108.

Cotoi, A. (2015). *Behavioral and Neural Correlates of Conditioned Pain Modulation in the Human Brainstem and Cervical Spinal Cord Using Functional Magnetic Resonance Imaging* (Doctoral dissertation).

Danielsson, I., Eisemann, M., Sjöberg, I., & Wikman, M. (2001). Vulvar vestibulitis: A multi-factorial condition. *BJOG: An International Journal of Obstetrics & Gynaecology*, 108(5), 456-461.

Davis, K. D., Kwan, C. L., Crawley, A. P., & Mikulis, D. J. (1998). Functional MRI study of thalamic and cortical activations evoked by cutaneous heat, cold, and tactile stimuli. *Journal of Neurophysiology*, *80*(3), 1533-1546.

Derbyshire, S. W. (2007). Imaging visceral pain. *Current Pain and Headache Reports*, 11(3), 178-182.

Derbyshire, S. W., Jones, A. K., Gyulai, F., Clark, S., Townsend, D., & Firestone, L. L. (1997). Pain processing during three levels of noxious stimulation produces differential patterns of central activity. *Pain*, 73(3), 431-445.

Dobek, C. E., Beynon, M. E., Bosma, R. L., & Stroman, P. W. (2014). Music modulation of pain perception and pain-related activity in the brain, brain stem, and spinal cord: a functional magnetic resonance imaging study. *The Journal of Pain*, 15(10), 1057-1068.

Downar, J., Mikulis, D. J., & Davis, K. D. (2003). Neural correlates of the prolonged salience of painful stimulation. *Neuroimage*, *20*(3), 1540-1551.

Drossman, D. A., Ringel, Y., Vogt, B. A., Leserman, J., Lin, W., Smith, J. K., & Whitehead, W. (2003). Alterations of brain activity associated with resolution of emotional distress and pain in a case of severe irritable bowel syndrome. *Gastroenterology*, 124(3), 754-761.

Edwards, R. R., Ness, T. J., Weigent, D. A., & Fillingim, R. B. (2003). Individual differences in diffuse noxious inhibitory controls (DNIC): association with clinical variables. *Pain*, 106(3), 427-437.

Figley, C. R., & Stroman, P. W. (2006). Characterization of spinal cord motion: a source of errors in spinal fMRI. In *Proceedings of the International Society for Magnetic Resonance in Medicine* (Vol. 14, pp. 4-9).

Foster, D. C., Dworkin, R. H., & Wood, R. W. (2005). Effects of intradermal foot and forearm capsaicin injections in normal and vulvodynia-afflicted women. *Pain*, *117*(1), 128-136.

Friston, K.J., Jezzard, P., Turner, R., (1994). Analysis of functional MRI time-series. *Human Brain Mapping* 1, 153–171.

Gebhart, G. F. (2004). Descending modulation of pain. *Neuroscience & Biobehavioral Reviews*, *27*(8), 729-737.

Giesecke, J., Reed, B. D., Haefner, H. K., Giesecke, T., Clauw, D. J., & Gracely, R. H. (2004). Quantitative sensory testing in vulvodynia patients and increased peripheral pressure pain sensitivity. *Obstetrics and Gynecology*, 104(1), 126–33.

Goldstein, A. T., Pukall, C. F., Brown, C., Bergeron, S., Stein, A., & Kellogg-Spadt, S. (2016). Vulvodynia: assessment and treatment. *The Journal of Sexual Medicine*, 13(4), 572-590.

Goetsch, M. F. (1991). Vulvar vestibulitis: prevalence and historic features in a general gynecologic practice population. *American Journal of Obstetrics & Gynecology*, *164*(6), 1609-1616.

Gracely, R. H., Petzke, F., Wolf, J. M., & Clauw, D. J. (2002). Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis & Rheumatism*, 46(5), 1333-1343.

Granot, M., Friedman, M., Yarnitsky, D., Tamir, A., & Zimmer, E. Z. (2004). Primary and secondary vulvar vestibulitis syndrome: systemic pain perception and psychophysical characteristics. *American journal of obstetrics and gynecology*, *191*(1), 138-142.

Granot, M., Friedman, M., Yarnitsky, D., & Zimmer, E. Z. (2002). Enhancement of the perception of systemic pain in women with vulvar vestibulitis. *BJOG: an International Journal of Obstetrics & Gynaecology*, *109*(8), 863-866.

Gupta, A., Kilpatrick, L., Labus, J., Tillisch, K., Braun, A., Hong, J. Y., ... & Mayer, E. A. (2014). Early adverse life events and resting state neural networks in patients with chronic abdominal pain: evidence for sex differences. *Psychosomatic Medicine*, 76(6), 404.

Gupta, A., Rapkin, A. J., Gill, Z., Kilpatrick, L., Fling, C., Stains, J., ... & Labus, J. S. (2015). Disease-related differences in resting state networks: a comparison between localized provoked vulvodynia, irritable bowel syndrome, and healthy control subjects. *Pain*, *156*(5), 809.

Halperin, R., Zehavi, S., Vaknin, Z., Ben-Ami, I., Pansky, M., & Schneider, D. (2005). The major histopathologic characteristics in the vulvar vestibulitis syndrome. *Gynecologic and Obstetric Investigation*, *59*(2), 75-79.

Hampson, J. P., Reed, B. D., Clauw, D. J., Bhavsar, R., Gracely, R. H., Haefner, H. K., & Harris, R. E. (2013). Augmented central pain processing in vulvodynia. *Journal of Pain*, 14(6), 579–589.

Harita, S., & Stroman, P. W. (2017). Confirmation of resting-state BOLD fluctuations in the human brainstem and spinal cord after identification and removal of physiological noise. *Magnetic resonance in medicine*, *78*(6), 2149-2156.

Harlow, B. L., Wise, L. A., & Stewart, E. G. (2001). Prevalence and predictors of chronic lower genital tract discomfort. *American Journal of Obstetrics and Gynecology*, 185(3), 545-550.

Hofbauer, R. K., Olausson, H. W., & Bushnell, M. C. (2006). Thermal and tactile sensory deficits and allodynia in a nerve-injured patient: a multimodal psychophysical and functional magnetic resonance imaging study. *The Clinical Journal of Pain*, 22(1), 104-108.

Hofbauer, R. K., Rainville, P., Duncan, G. H., & Bushnell, M. C. (2001). Cortical representation of the sensory dimension of pain. *Journal of Neurophysiology*, *86*(1), 402-411.

Jensen, K. B., Loitoile, R., Kosek, E., Petzke, F., Carville, S., Fransson, P., ... & Vitton, O. (2012). Patients with fibromyalgia display less functional connectivity in the brain's pain inhibitory network. *Molecular pain*, *8*(1), 32.

Jodoin, M., Bergeron, S., Khalifé, S., Dupuis, M. J., Desrochers, G., & Leclerc, B. (2008). Male partners of women with provoked vestibulodynia: Attributions for pain and their implications for dyadic adjustment, sexual satisfaction, and psychological distress. *The Journal of Sexual Medicine*, *5*(12), 2862-2870.

Johannesson, U., de Boussard, C. N., Jansen, G. B., & Bohm-Starke, N. (2007). Evidence of diffuse noxious inhibitory controls (DNIC) elicited by cold noxious stimulation in patients with provoked vestibulodynia. *Pain*, *130*(1), 31-39.

Khan, H. S., & Stroman, P. W. (2015). Inter-individual differences in pain processing investigated by functional magnetic resonance imaging of the brainstem and spinal cord. *Neuroscience*, 307, 231-241.

Kuchinad, A., Schweinhardt, P., Seminowicz, D. A., Wood, P. B., Chizh, B. A., & Bushnell, M. C. (2007). Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain?. *Journal of Neuroscience*, 27(15), 4004-4007.

Lautenbacher, S., Kunz, M., Strate, P., Nielsen, J., & Arendt-Nielsen, L. (2005). Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain. *Pain*, *115*(3), 410-418.

Lautenbacher, S., & Rollman, G. B. (1997). Possible deficiencies of pain modulation in fibromyalgia. *The Clinical Journal of Pain*, *13*(3), 189-196.

Mayer, E. A., Labus, J. S., Tillisch, K., Cole, S. W., & Baldi, P. (2015). Towards a systems view of IBS. *Nature Reviews Gastroenterology and Hepatology*, *12*(10), 592.

Melzack, R., & Katz, J. (2007). McGill pain questionnaire. In *Encyclopedia of Pain* (pp. 1102-1104). Springer Berlin Heidelberg.

Millan, M. J. (2002). Descending control of pain. *Progress in neurobiology*, *66*(6), 355-474.

Molnar-Szakacs, I., & Uddin, L. Q. (2013). Self-processing and the default mode network: interactions with the mirror neuron system. Frontiers in human neuroscience, 7, 571.

Myronenko, A., & Song, X. (2009, June). Image registration by minimization of residual complexity. In *Computer Vision and Pattern Recognition, 2009. CVPR 2009. IEEE Conference on* (pp. 49-56). IEEE.

Myronenko, A., & Song, X. (2010). Intensity-based image registration by minimizing residual complexity. *IEEE Transactions on Medical Imaging*, *29*(11), 1882-1891.

Nguyen, R. H., Veasley, C., & Smolenski, D. (2013). Latent class analysis of comorbidity patterns among women with generalized and localized vulvodynia: preliminary findings. *Journal of pain research*, *6*, 303.

Normand, E., Potvin, S., Gaumond, I., Cloutier, G., Corbin, J. F., & Marchand, S. (2011). Pain inhibition is deficient in chronic widespread pain but normal in major depressive disorder. *The Journal of Clinical Psychiatry*, 72(2), 219-224.

Oertel, B. G., Preibisch, C., Martin, T., Walter, C., Gamer, M., Deichmann, R., & Lötsch, J. (2012). Separating brain processing of pain fromthat of stimulus intensity. *Human brain mapping*, *33*(4), 883-894.

Olausson, H., Ha, B., Duncan, G. H., Morin, C., Ptito, A., Ptito, M., ... & Bushnell, M. C. (2001). Cortical activation by tactile and painful stimuli in hemispherectomized patients. *Brain*, *124*(5), 916-927.

Oshiro, Y., Quevedo, A. S., McHaffie, J. G., Kraft, R. A., & Coghill, R. C. (2009). Brain mechanisms supporting discrimination of sensory features of pain: a new model. *Journal of Neuroscience*, *29*(47), 14924-14931.

Ossipov, M. H., Morimura, K., & Porreca, F. (2014). Descending pain modulation and chronification of pain. *Current opinion in supportive and palliative care*, *8*(2), 143.

Pazmany, E., Ly, H. G., Aerts, L., Kano, M., Bergeron, S., Verhaeghe, J., ... & Van Oudenhove, L. (2017). Brain responses to vestibular pain and its anticipation in women with Genito-Pelvic Pain/Penetration Disorder. *NeuroImage: Clinical*, *16*, 477-490.

Piché, M., Arsenault, M., & Rainville, P. (2009). Cerebral and cerebrospinal processes underlying counterirritation analgesia. *Journal of Neuroscience*, *29*(45), 14236-14246.

Pukall, C. F. (2016). Primary and secondary provoked vestibulodynia: a review of overlapping and distinct factors. *Sexual medicine reviews*, *4*(1), 36-44.

Pukall, C. F., & Cahill, C. M. (2014). New developments in the pathophysiology of genital pain: Role of central sensitization. *Current Sexual Health Reports*, *6*(1), 11-19.

Pukall, C. F., Goldstein, A. T., Bergeron, S., Foster, D., Stein, A., Kellogg-Spadt, S., & Bachmann, G. (2016). Vulvodynia: definition, prevalence, impact, and pathophysiological factors. *The journal of sexual medicine*, *13*(3), 291-304.

Pukall, C. F., Baron, M., Amsel, R., Khalifé, S., & Binik, Y. M. (2006). Tender point examination in women with vulvar vestibulitis syndrome. *The Clinical Journal of Pain*, *22*(7), 601-609.

Pukall, C. F., Binik, Y. M., Khalifé, S., Amsel, R., & Abbott, F. V. (2002). Vestibular tactile and pain thresholds in women with vulvar vestibulitis syndrome. *Pain*, 96(1–2), 163–175.

Pukall, C. F., Strigo, I. A., Binik, Y. M., Amsel, R., Khalifé, S., & Bushnell, M. C. (2005). Neural correlates of painful genital touch in women with vulvar vestibulitis syndrome. *Pain*, 115(1–2), 118–127.

Reed, B. D., Harlow, S. D., Sen, A., Edwards, R. M., Chen, D., & Haefner, H. K. (2012). Relationship between vulvodynia and chronic comorbid pain conditions. *Obstetrics and Gynecology*, 120(1), 145.

Rosen, C. Brown, J. Heiman, S. Leiblum, C. Meston, R. Shabsigh, D. Ferguson, R. D'Agostino, R. (2000). The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. *Journal of Sex & Marital Therapy*, *26*(2), 191-208.

Schmidt-Wilcke, T., Leinisch, E., Straube, A., Kämpfe, N., Draganski, B., Diener, H. C., ... & May, A. (2005). Gray matter decrease in patients with chronic tension type headache. *Neurology*, *65*(9), 1483-1486.

Schweinhardt, P., Kuchinad, A., Pukall, C. F., & Bushnell, M. C. (2008). Increased gray matter density in young women with chronic vulvar pain. *Pain*, 140(3), 411–419.

Smith, K. B., & Pukall, C. F. (2014). Sexual function, relationship adjustment, and the relational impact of pain in male partners of women with provoked vulvar pain. *The journal of sexual medicine*, *11*(5), 1283-1293.

Spielberger, C. D. (2010). *State-Trait anxiety inventory*. John Wiley & Sons, Inc..

Sprenger, C., Bingel, U., & Büchel, C. (2011). Treating pain with pain: supraspinal mechanisms of endogenous analgesia elicited by heterotopic noxious conditioning stimulation. *PAIN*, *152*(2), 428-439.

Staud, R. (2013). The important role of CNS facilitation and inhibition for chronic pain. *International journal of clinical rheumatology*, *8*(6), 639.

Stroman, P. W. (2009). Spinal fMRI investigation of human spinal cord function over a range of innocuous thermal sensory stimuli and study-related emotional influences. *Magnetic resonance imaging*, *27*(10), 1333-1346.

Stroman, P. W. (2011). Investigating pain networks in the spinal cord using functional Mri. *Imaging in Medicine*, *3*(2), 141.

Stroman, P. W. (2016). Validation of Structural Equation Modeling Methods for Functional MRI Data Acquired in the Human Brainstem and Spinal Cord. *Critical Reviews™ in Biomedical Engineering*, *44*(4).

Stroman, P. W., Bosma, R. L., Cotoi, A. I., Leung, R. H., Kornelsen, J., Lawrence-Dewar, J. M., ... & Staud, R. (2016). Continuous descending modulation of the spinal cord revealed by functional MRI. *PLoS One*, 11(12), e0167317.

Stroman, P. W., Ioachim, G., Powers, J. M., Staud, R., & Pukall, C. (2018). Pain processing in the human brainstem and spinal cord before, during, and after the application of noxious heat stimuli. *Pain*.

Stroman, P. W., Khan, H. S., Bosma, R. L., Cotoi, A. I., Leung, R., Cadotte, D. W., & Fehlings, M. G. (2016). Changes in pain processing in the spinal cord and brainstem after spinal cord

injury characterized by functional magnetic resonance imaging. *Journal of Neurotrauma*, *33*(15), 1450-1460.

Stroman, P. W., Wheeler-Kingshott, C., Bacon, M., Schwab, J. M., Bosma, R., Brooks, J., ... & Curt, A. (2014). The current state-of-the-art of spinal cord imaging: methods. *Neuroimage*, *84*, 1070-1081.

Sullivan, M. J., Bishop, S. R., & Pivik, J. (1995). The pain catastrophizing scale: development and validation. *Psychological Assessment*, 7(4), 524.

Sutton, K. S., Pukall, C. F., & Chamberlain, S. (2009). Pain ratings, sensory thresholds, and psychosocial functioning in women with provoked vestibulodynia. *Journal of sex & marital therapy*, *35*(4), 262-281.

Sutton, K., Pukall, C., Wild, C., Johnsrude, I., & Chamberlain, S. (2015). Cognitive, psychophysical, and neural correlates of vulvar pain in primary and secondary provoked vestibulodynia: a pilot study. *The journal of sexual medicine*, *12*(5), 1283-1297.

Sutton, K. S., Pukall, C. F., & Chamberlain, S. (2012). Diffuse noxious inhibitory control function in women with provoked vestibulodynia. *The Clinical Journal of Pain*, *28*(8), 667-674.

Villemure, C., & Bushnell, C. M. (2002). Cognitive modulation of pain: how do attention and emotion influence pain processing?. *Pain*, *95*(3), 195-199.

Vincent, K., & Tracey, I. (2008). Hormones and their Interaction with the Pain Experience. *Reviews in Pain*, *2*(2), 20–24.

Wheeler-Kingshott, C. A., Stroman, P. W., Schwab, J. M., Bacon, M., Bosma, R., Brooks, J., ... & Curt, A. (2014). The current state-of-the-art of spinal cord imaging: applications. *Neuroimage*, *84*, 1082-1093.

White, K. P., & Harth, M. (2001). Classification, epidemiology, and natural history of fibromyalgia. *Current pain and headache reports*, *5*(4), 320-329.

Wilson, H., & Tashani, O. A. (2016). Hormonal contraceptive pill effect on pain sensitivity response. *International Journal of Clinical and Experimental Physiology*, *3*(4), 166.

Yen, C. T., & Lu, P. L. (2013). Thalamus and pain. *Acta Anaesthesiologica Taiwanica*, 51(2), 73-80.

Appendix A: Queen's University Research Ethics Board Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD (HSREB)

HSREB Initial Ethics Clearance

March 14, 2016

Dr. Caroline Pukall
Department of Psychology
Humphrey Hall

ROMEO/TRAQ: #6017470
Department Code: PSYC-170-16
Study Title: Investigation of descending pain modulation in the brain and spinal cord of women with vulvodynia
Co-Investigators: Dr. S. Chamberlain, Dr. P. Stroman
Review Type: Full Board
Date of Full Board Meeting: February 8, 2016
Date Ethics Clearance Issued: March 14, 2016
Ethics Clearance Expiry Date: March 14, 2017

Dear Dr. Pukall,

The Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the application and granted ethics clearance for the documents listed below. Ethics clearance is granted until the expiration date noted above.

- Protocol
- References
- Budget
- Reproductive Risks Explanation
- Validated Measures
- Sexual Health Research Laboratory Terms of Agreement and Confidentiality Statement
- Recruitment Advertisements
- Telephone Screening Interview
- Gynaecological Examination Sheet
- Interview Guide – Version 2
- Debriefing Form – Version 2 – 2016/02/19
- Letter of Information/Consent Form – Version 2- 2016/02/19

Amendments: No deviations from, or changes to the protocol should be initiated without prior written clearance of an appropriate amendment from the HSREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of

the trial.

Renewals: Prior to the expiration of your ethics clearance you will be reminded to submit your renewal report through ROMEO. Any lapses in ethical clearance will be documented on the renewal form.

Completion/Termination: The HSREB must be notified of the completion or termination of this study through the completion of a renewal report in ROMEO.

Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.

Reporting of Complaints: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete.

Yours sincerely,

Albert J. Clark.

Chair, Health Sciences Research Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations, Canadian General Standards Board, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federalwide Assurance Number: FWA#:00004184, IRB#:00001173

HSREB members involved in the research project do not participate in the review, discussion or decision.

Appendix B: Letter of Information and Consent

Invitation to participate in a research study.

Title: *Investigation of descending pain modulation in the brain and spinal cord of women with vulvodynia*

Letter of Information

Investigators:
Caroline F. Pukall, PhD, Department of Psychology, Queen's University
Patrick Stroman, PhD, Centre for Neuroscience Studies, Queen's University
Susan M. Chamberlain, MD, Department of Obstetrics & Gynecology, Kingston General Hospital
Linda Booij, Department of Psychology, Concordia University
Moshe Szyf, Department of Pharmacology and Therapeutics, McGill University
Lindsey Yessick, MSc Student, Department of Psychology, Queen's University
Gabriela Ioachim, MSc Student, Centre for Neuroscience Studies, Queen's University

Purpose of the study
The purpose of this study is to investigate how your brain and spinal cord/brainstem perceive pain, and to examine how DNA relates to pain. The current study is a research project being conducted by Drs. Caroline Pukall, Patrick Stroman, Susan Chamberlain, Linda Booij, and Moshe Szyf, and graduate students Lindsey Yessick and Gabriela Ioachim. This study will examine differences in pain processing in four groups of women: (1) women with provoked vestibulodynia (PVD) (i.e., provoked genital pain at the vaginal opening, a common cause of painful intercourse); (2) women with generalized vulvodynia (GVD) (i.e., genital pain that is always or almost always present over the entire vulva and that can be made worse by engaging in activities such as walking); (3) women with fibromyalgia (i.e., chronic, generalized pain and tenderness, and associated fatigue); and (4) pain-free control women (i.e., women who are free of genital pain, fibromyalgia, and other chronic pain conditions). Measures of psychosocial functioning (e.g., depression) will also be examined to assess whether or not they are related to the experience of pain in terms of the intensity and unpleasantness of the pain. In addition, your DNA will be examined to see how it relates to pain and the measures of psychosocial functioning.

Study procedures
Your participation in this study is voluntary and you are free to withdraw at any time.

Should you choose to participate, and you have **vulvodynia**, this involves undergoing the following procedures: 1) a telephone/online screening questionnaire; 2) a gynecological examination to confirm eligibility; 3) online (or hard copy) completion of questionnaires; 4) a sensory testing/sham fMRI session, followed by a saliva collection, and the first functional MRI (fMRI) imaging session; and 5) a second fMRI imaging session.

Should you choose to participate, and you have **fibromyalgia** or **no chronic pain condition**, this involves undergoing the following procedures: 1) a telephone/online screening questionnaire to determine eligibility; 2) online (or hard copy) completion of questionnaires; 3) a sensory

testing/sham fMRI session, followed by a saliva collection, and the first functional MRI (fMRI) imaging session; and 4) a second fMRI imaging session.

The telephone/online screening questionnaire to determine eligibility includes questions about sociodemographics (e.g., age, relationship status), medical history (e.g., diagnoses received, medications, health), study-specific information (e.g., whether you have any contraindications for the imaging sessions such as current pregnancy or metal implants), and pain characteristics (e.g., location, duration). At the end of this questionnaire, you will find out whether or not you are eligible for the study.

If you are eligible and you have **vulvodynia**, the gynecological examination will take place at the Department of Psychology, Queen's University and will require you to be present for approximately 30 minutes, 5 to 10 of which will consist of the actual gynecological examination.

If you are eligible and you have **vulvodynia**, **fibromyalgia**, or **no pain**, the completion of questionnaires will take place online, on a secure website, or you can opt to receive hard copies of the questionnaires via mail. If completed online, questionnaires should be completed before the first imaging session. If completed at home, please bring the questionnaires with you to the first imaging session. The sensory testing/sham fMRI and first imaging session will take place at the Queen's MRI Facility located in the Cancer Research Institute at 15 O'Kill Street. This first session will take approximately 2.5 hours to complete. The second session will take place at the same location, and will take approximately 1.5 hour to complete. Note that you will be asked to refrain from alcohol for at least 12 hours prior to the imaging portions of the study, and caffeine for at least 6 hours before the study. You will also be asked to ensure that you eat a regular meal at your usual meal time before each study session.

(FOR WOMEN WITH VULVODYNIA ONLY) Gynecological examination: During the gynecological examination (5-10 minutes), the doctor will visually and manually examine your internal and external genitalia and reproductive organs and will touch different areas of your vulva (i.e., external genitals) with a cotton-swab. The doctor will ask you to rate any pain you experience during this examination on a scale of 0 to 10. You will be in complete control of the procedure and may ask to stop at any time and/or control the speed of the examination. A research assistant will be, and a medical student may be, present during the examination.

(FOR ALL PARTICIPANTS) Questionnaires: The online/hard copy questionnaires will take approximately 45 minutes to complete and will cover detailed sociodemographic information (e.g., age, culture, gender, sexual orientation, income, relationship status), gynecological and medical history, vulvar/fibromyalgia pain history, current physical and psychological symptoms, and sexual functioning. If you opt to receive the questionnaires via mail, then you mailing address will be required.

Sensory testing session: During the sensory testing session, a square temperature device (probe) will be placed on your hand and it will administer different levels of warmth and heat. Since we are interested in seeing how your brain and spinal cord/brainstem perceive pain, we will choose a level of temperature that is moderately painful for you, for the brain imaging session. We will choose that level based on your ratings of the sensations on different scales, one for the intensity of the sensation, and one for the unpleasantness of the sensation.

Although some of the temperature levels in the sensory testing session may feel uncomfortable or painful to you, none will damage your skin. We will also be applying a pressure device (algometer) to measure tender areas on certain locations of your body. You can withdraw from and terminate any stimulus that is too uncomfortable at any time. This portion of the study will take approximately 1 hour to complete. It takes place in the "Sham" MRI room at the Queen's MRI Facility. The "sham" MRI is a mock-up of the actual MRI system which provides you with the experience of undergoing MRI, without the magnetic field. We will practice the experiment in the sham MRI to allow you to become familiarized with the environment and QST procedures.

Saliva sample: You will be asked to contribute 1 ml of saliva into a 3-6 ml tube by passive drooling in order to provide DNA material. This procedure will be done after a period of 30 minutes during which there is no eating or drinking.

Brain imaging session #1: Before the imaging session, you will begin by filling out a checklist and questionnaire to make sure you are eligible (5 minutes). If you are pregnant or are trying to conceive, you will not be eligible. If there is any uncertainty regarding whether or not you are pregnant and you want to participate in the study, a pregnancy test must be done prior participating in the study.

During the brain imaging session, one resting-state scan (6 minutes), 10 functional scans (4.5 minutes each), and then an anatomical scan (7 minutes) will be conducted, either of your brain or your spinal cord/brainstem region. The area to be scanned will be randomly decided upon and the area not scanned in the first session will be scanned in the second session (see below). During the anatomical scan, the scanner will take a 3-dimensional picture of your brain (or spinal cord/brainstem region), and the resting-state scan is like a functional scan as you rest. Half of the functional scans will involve you simply resting, and the other half will involve the application of the moderately painful stimulation to your hand alternating with periods of no stimulation. The order of the scans will be randomized, and you be told what to expect (rest or stimulation) during each scan.

Brain imaging session #2: During the second brain imaging session, the same brain imaging procedure as described above will take place. The only difference will be the scanned region (either brain or spinal cord/brainstem region).

Brain imaging information

If you agree to participate, either your brain or spinal cord will be imaged while you are lying in a 3 Tesla magnetic resonance imaging (MRI) scanner in the Queen's fMRI Facility, and your heart beat and breathing may be monitored. The entire session may last up to 2.5 hours including getting ready for the study and positioned in the magnet etc. This study involves a single visit to the lower level of the Cancer Research Institute for imaging.

a) You will begin by filling out a checklist and questionnaire to make sure you are eligible. This will be completed first, and will take about 5 minutes. If you are currently breastfeeding, pregnant or are trying to conceive you will not be eligible. If there is any uncertainty regarding whether or not you are pregnant and you want to participate in the study, a pregnancy test must be done prior to the experiment.

b) Prior to the scan, the square probe for the sensory testing session] that will be used for brain imaging studies will be shown to you, used to administer different levels of warmth and heat to your hand] for fMRI studies.

c) Please try to wear clothing containing no metal, or to bring a change of clothing. Metal in zippers, snaps, and the wire and metal clasps in some bras can interfere with the imaging. Many shoes contain metal as well. You will be asked to remove or change out of any clothes that contain metal that will be near the area being imaged, and you will be asked to remove your shoes. For imaging the brain and upper portion of the spinal cord, the snaps and zippers in jeans or other pants are far enough from the area being imaged that they do not cause a problem.

d) You will be asked to wear disposable earplugs to protect your ears from the noise of the MRI system. You will still be able to hear the researchers over the two-way communication system with these earplugs in place.

e) You will be asked to lie on your back on the well-padded bed of the magnet. Pillows will be placed under your legs for comfort and a blanket will be placed over your legs if you wish.

f) For brain imaging studies, a head coil will be placed over your head. This is fitted with a mirror so that you can see out of the magnet towards your head or feet. For spinal cord imaging studies you will lay on top of a flat spine coil that looks like part of the bed you are lying on with a head support. You and the bed will then slide into a long tube (the magnet).

g) You will need to keep still while the images are taken. To help you, we will make you as comfortable as possible and we will pack soft foam around your head if needed.

h) The MR system has a two-way intercom for communication. During the imaging to map the function in your brain you will feel changes in temperature from the small thermode placed on your hand and you will be asked to rate the sensations on intensity and unpleasantness scales.

i) At the end of the session, additional images will be taken of the anatomy (or structure) of your brain or spinal cord.

The MRI scanning procedure is very much like other medical imaging used in hospitals, but you will not be exposed to x-rays. This MRI machine uses a strong magnet and radio waves to make images of the interior of your body. You will not feel either. The MRI being used in this study is a 3 Tesla MRI that is twice that used for most clinical imaging, although 3 tesla systems are becoming more common in hospitals. The levels of magnetism and radio waves used in the MRI have not been shown to cause harmful effects. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic objects must be removed from your person before you approach the scanner. If you have a cardiac pacemaker or a metallic clip in your body (e.g., an aneurysm clip in your brain or an I.U.D.) you should not participate in any MRI study. In addition, credit cards and other cards with magnetic strips should also be removed as these will be damaged. (These items will be kept safe for you).

You will be in voice contact with the operator, and the operator will be able to see you via a camera. You may ask the operator to stop the experiment at any time. You should ask to stop the experiment if you feel tired, claustrophobic, or uncomfortable.

Advantages of participating in this study

You will not get a personal medical benefit from participating in this study but your participation will help us to improve functional imaging of the brain and spinal cord. The information obtained from this study will potentially help our understanding of the spinal, brain, and DNA processes involved in the development and maintenance of vulvodynia and fibromyalgia and, possibly, other related chronic pain conditions.

Disadvantages of participating in this study

The major disadvantage involved in participating in this study is that some of the above procedures (i.e., gynecological examination, and sensory testing and fMRI sessions) may be uncomfortable or painful. The pressure stimuli used during the sensory testing session are intended to cause pain, and may result in discomfort, and/or temporary reddening of the skin, but they will not damage your skin. In addition, as some of questionnaires cover sensitive topics, such as anxiety and sexual functioning, you may experience some discomfort answering them. You may also not feel comfortable providing a saliva sample. Remember that you are free to discontinue your participation or opt out of any portion of the study at any point during the study without having to provide explanation.

There are no known risks involved with magnetic resonance imaging. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic or magnetic objects must be removed from your person before you approach the scanner.

MRI may be dangerous for anyone with metal inside their body. Some metal objects may move or heat up due to the magnetic force and radiofrequency waves used for MRI. We will screen the participants to make sure that it is safe for them to participate (please see screening form).

No long-term adverse effects of MRI have been reported in either adults or children. Nonetheless, women who are, or who may be pregnant, will be excluded from this study.

MRI is completely painless, but some people have felt minor, transient discomforts during MRI scans (e.g. dizziness, lightheadedness or a feeling of continued motion after being moved into the magnetic field), which usually subside within a few minutes. In rare cases, the dizziness progresses to the point of nausea, but subsides quickly outside the magnetic field. Some people may have a feeling of claustrophobia while they are in the scanner, and in extremely rare cases, this feeling seems to have triggered a more persistent claustrophobia. No long-term adverse effects of MRI have been reported.

The following specific risks have been identified and are included in the screening form:

<u>Previous Surgery, such as:</u>

- Surgery involving metal, such as: clips, rods, screws, pins, wires.
- Heart pacemaker
- Implanted electrodes, pumps or electrical devices
- Cochlear (inner ear) implants

- Intraocular lens (eye) implants (Cataract lens allowed except for Brain Imaging studies)

Any metallic foreign body, shrapnel or bullet – people who have worked as a grinder, metal worker, welder, or have been wounded during military service, will be excluded

- Intrauterine contraceptive device (IUD) or contraceptive diaphragm
- Dental work held in place by magnets
- Non-removable dental braces and retainers
- Tattooed eyeliner
- Some tattoos (particularly recent tattoos and/or containing black ink)
- Non-removable metal jewelry (body piercing)

Exclusion criteria

Due to the very high magnetic field you should not be a participant in any MRI experiment if you…: (any of the following)

1. have a history of head or eye injury involving metal fragments.
2. have ever worked in a metal shop
3. have some type of implanted electrical device (such as a cardiac pacemaker or neurostimulator)
4. have implanted metal objects as a result of surgery such as artificial joints, aneurysm clips, metal staples
5. have severe heart disease (including susceptibility to arrhythmias) or any other serious illness
6. are, or may be, pregnant
7. are wearing metal braces on your teeth
8. have non-removable jewelry (body piercing)

Confidential nature of this study

The findings of this study will be reported in scientific journals but your name will remain confidential. Data from your images will be stored on a secure computer system and identified only with the date and a participant code. Only the researchers directly related to this study will have access to the data files and the participant codes. You will not be identified in any publication or reports. All written records and data will be identifiable only by your participant ID number, and all of this information will be kept in a locked filing cabinet. You will not be identified in any publication or reports of this research; data will be aggregated in all reports of this study.

Although this is not a diagnostic scan and any images obtained are for research purposes only, it is possible that the MR scan may disclose an unknown abnormality. In this event, a medical imaging specialist will be asked to review the images and we would send a report to your physician. The researchers directly involved with this procedure do not have the credentials to diagnose medical conditions.

Each participant will be assigned a participant number. This number will be used for all studies, should they choose to participate in other studies being carried out in the Queen's MRI Facility. This common number is to enable records of total time exposed to the magnetic field to be maintained. This is to protect the participants in the event that there should ever be any evidence found that exposure to magnetic fields of 3 tesla can pose a health risk. This will also enable the Facility to maintain a record of the safety checklists completed by a given person for each study

in which they participate. This information will be stored in a locked filing cabinet, with files identified only by the participant number. A record of the participant number that has been assigned to each person will be maintained on a secure password-protected computer in the MRI Facility that is not connected to any networks. This file will not be accessible to any researchers including the PI, but can only be accessed by the MR Technologist and Facility Director for purposes of maintaining safety records. Upon request, the PI will be able to obtain a record of the participant names and corresponding participant numbers for those who participated in this study.

The participant's weight and age (not birth date) will be recorded. The person's weight is required for safety monitoring during the MR imaging, because the MR system ensures that the amount of energy deposited in the body does not exceed FDA or Health Canada guidelines. The person's age is required so that the group of volunteers can be characterized when reporting the results. The participant number, weight, and age, will be the only identifying information attached to the MR image data or notes taken during the study. The data will therefore be de-identified. The screening form and consent form that will be signed by the volunteer prior to participating, and the file linking the person's name to the participant number, will be the only records linking the participant's name to their assigned participant number. The screening and consent forms will be stored in a locked filing cabinet in the P.I.'s office. After five years these records will be disposed of by shredding. The P.I. will be responsible for all data monitoring and analysis.

DNA samples will be securely stored in Dr. Pukall's office until analyses are ready to take place. DNA analyses will take place in the laboratory of Dr. Szyf (McGill University, Department of Pharmacology and Therapeutics). Participant samples will be identified by participant code only. Dr. Szyf and his team will not have access to any personal information (e.g., names) of any participant. DNA can be used for future related studies conducted by Dr. Pukall and her team for 10 years after the start of this study if you give special permission to do so. All DNA will be destroyed after 10 years (October 2026). It is important to note that advancing technology may lead to the possibility in the future that genetic information could lead to identification

Withdrawal of participant by principal investigator

The study Director may decide to withdraw you from this study if:

1) you do not meet the criteria in the Magnetic Resonance Screening Form.
2) you do not meet the eligibility criteria for the study groups

Discontinuation of this study

You are under no obligation to participate in this study, and your acceptance or refusal will not affect access to services. Furthermore, you are free to withdraw from the study at any time, and you are free to refuse to answer any question posed without need of any explanation on your part. In addition, the investigators may end your participation in this study for purely scientific reasons at any time.

Any form of verbal and/or non-verbal sexual misconduct (e.g., sexual advances, sexual innuendo, physical contact with the experimenter) will result in the immediate termination of your participation in the study. A report will be made to the Sexual Violence Prevention and Response Coordinator, Barb

Lotan, as per University Policy. Additionally, the University may file a criminal police report with local authorities.

Liability

In the event that you are injured as a result of the study procedures, medical care will be provided to you until resolution of the medical problem. By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

Compensation

If you are a woman with vulvodynia, upon completion of the gynecological examination, the questionnaires, and the sensory testing, DNA sample, and first and second fMRI sessions, you will receive $75.00 as compensation for your time and inconvenience. If you are a woman with fibromyalgia or no chronic pain condition, upon completion of the questionnaires, and the sensory testing, DNA sample, and first and second fMRI sessions, you will receive $60.00 as compensation for your time and inconvenience. If the study has to be terminated for scientific reasons or if you decide to terminate the study, compensation will be adjusted according to the fraction of the study completed.

First Nations Elders

If you are a First Nations person, or an indigenous person who has contact with spiritual elders, you may want to talk with them before you proceed with being part of this experiment. Elders have reservations about genetic procedures.

Consent

I, _____, have volunteered to participate in the study entitled *Investigation of descending pain modulation in the brain and spinal cord of women with vulvodynia by* Drs. Caroline Pukall, Patrick Stroman, Linda Booij, Moshe Szyf, and Susan Chamberlain, and Master's graduate students Lindsey Yessick and Gabriela Ioachim.

I understand that, if I have genital pain, I will undergo a gynecological examination that will take place at the Department of Psychology (Queen's University) to determine what genital pain problem I have. If I have fibromyalgia or am pain-free, or if I have vulvodynia as determined by the gynecological examination, I understand that I will complete questionnaires online or in hard copy. I understand that some of the questions in this session may be quite personal in nature as some of them are related to topics such as anxiety or sexual functioning. Further, I understand that I will provide a saliva sample for the purpose of DNA analysis, and will undergo a sensory testing session and two functional magnetic resonance imaging (fMRI) sessions which involve the application of a square probe placed on my hand and it will administer different levels of warmth and heat. I understand that my participation in the study is completely voluntary and that I am free to withdraw at any time. I also understand that my confidentiality will be protected throughout the study, and that the information I provide will be available to researchers with scholarly interests in vulvodynia (i.e., chronic genital pain in women) and fibromyalgia (chronic widespread pain and tenderness with associated symptoms such as fatigue). Lastly, I understand that a medical imaging specialist will be asked to review the brain scan images and I consent that a report can be sent to the physician I list below.

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I chose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I have named Dr. _____ at _____ as the physician to be contacted for follow-up purposes. I am voluntarily signing this form. I will receive a copy of this consent form for my information. If at any time I have further questions, problems or adverse events, I can contact:

Dr. Patrick Stroman	Dr. Caroline Pukall	Dr. Wendy Craig
Principal Investigator	Principal Investigator	Department Head (Psychology)
Queen's University	Queen's University	Queen's University
Kingston, Ontario	Kingston, Ontario	Kingston, Ontario
K7L 3N6	K7L 3N6	K7L 3N6
Phone: 613-533-3245	613-533-3200	613-533-6014
stromanp@queensu.ca	caroline.pukall@queensu.ca	wendy.craig@queensu.ca

If you have any concerns about your rights as a research participant please contact - Dr. Albert Clark, Chair of the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at 1-844-535-2988.

By signing this consent form, I am indicating that I agree to participate in:

__ The gynecological examination (if I have vulvodynia)

__ The questionnaires

__ The sensory testing portion of the study

__ The collection of DNA using saliva

__ The imaging portion of the study (fMRI)

__ AND I agree that DNA will be stored under a code and can be used for future related studies done by Dr. Pukall and her team. Any new use of DNA will undergo ethics review and approval first.

_____ _____

Signature of Volunteer Date

_____ _____

Signature of Witness Date

STATEMENT OF INVESTIGATOR:

I, or one of my colleagues, have carefully explained to the participant the nature of the above research study. I certify that, to the best of my knowledge, the participant understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

_____ _____

Signature of Principal Investigator Date

Information about Confidentiality

All information disclosed during your participation in this research study is confidential and **will not** be disclosed to anyone without your written and informed consent **except** where reporting is required by law, that is:

1. Where there is suspicion that a child or children (that is, an individual who is PRESENTLY under the age of 16) has been or is being abused;

2. Where the research participant is likely to harm herself or himself unless protective measures are taken;

3. Where the research participant presents a serious danger of violence to others, and;

4. If the research participant reveals that she has been sexually abused by a health care provider (for example, a psychologist or physician) covered by the Regulated Health Professionals Act, it is necessary by law to report the name of the perpetrator to his/her governing body.

IF YOU HAVE ANY CONCERNS ABOUT THESE MATTERS, OR ABOUT THIS FORM, PLEASE DISCUSS THESE WITH DR. CAROLINE PUKALL.

****

PLEASE SIGN THE ACKNOWLEDGEMENT BELOW TO INDICATE THAT YOU HAVE READ THIS INFORMATION ABOUT CONFIDENTIALITY

****

I acknowledge the circumstances that limit confidentiality and I accept them.

_____ _____ _____

Participant's name Participant's signature Date

_____ _____ _____

Witness' name Witness' signature Date

Interest in Future Studies

The Sexual Health Research Lab conducts a number of studies relating to sexual heath and functioning. Should you wish, we can keep your contact information in a password-protected participant recruitment file so that you can be contacted by a member of our research team for participation in other relevant studies.

- ❑ **Yes**, please contact me with information about future studies

Contact Information:

Name (please print):_____

Email:_____

Phone Number:_____

- ❑ **No**, please do not contact me regarding future research

Appendix C: Gynecological Exam

Medical History

Age: _____ Weight: _____ Height: _____

Major illnesses & hospitalizations

Cardiovascular/vascular (e.g., angina, heart attack, transient ischemic attack, stroke, etc)

_____

Respiratory (e.g., asthma, chronic obstructive pulmonary disease, emphysema, etc)

_____

Gastrointestinal/renal (e.g., irritable bowel syndrome, interstitial cystitis, etc)

_____

Musculoskeletal/rheumatological (e.g., fibromyalgia, arthritis, etc)

_____

Endocrinological (e.g., hypothyroidism, diabetes, etc)

_____

Gynecological (e.g., endometriosis, pelvic inflammatory disease, recurrent yeast infections, etc)

_____

Psychiatric/psychological (e.g., depression)

_____

_____

Past surgeries

_____

_____

Reproductive history

Number of pregnancies: _____

Number of live births: _____

Mode of delivery: _____

Pregnancy complications: _____

_____

Sexually transmitted infections (e.g., Chlamydia, gonorrhea, herpes, HPV)

_____

Current medications

_____

_____

Allergies

_____

_____

Other

_____

_____

_____

_____

GYNECOLOGICAL EXAMINATION

Participant ID Number: _____ Date: _____

Examining physician: _____Research Assistant: _____

SECTION I: INSPECTION OF THE VULVA

Clitoris

No abnormalities ☐	Partially hooded ☐	Complete phimosis ☐

Labia minora

No abnormalities ☐	Partially fused ☐	Completely fused ☐

Posterior fourchette

No abnormalities ☐	Scar from previous fissure ☐	Active fissure ☐

Vestibule: BEFORE the cotton-swab test

No abnormalities ☐	Erythema ☐	Fissure ☐	Synechia ☐

Pubic Hair

Sparse ☐	Normal ☐	Shaved ☐

Labia

Dry, atrophic ☐	In between atrophic and full ☐	Full ☐

SECTION II: COTTON-SWAB TEST

LABIA MAJORA: pain intensity ratings

Patient's right, anterior: _____ Patient's left, anterior: _____

Patient's right, mid-point: _____ Patient's left, mid-point: _____

Patient's right, posterior: _____ Patient's left, posterior: _____

INNER LABIA MINORA: pain intensity ratings

Patient's right, anterior: _____ Patient's left, anterior: _____

Patient's right, posterior: _____ Patient's left, posterior: _____

MIDLINE AREAS and VESTIBULE (random order): pain intensity ratings

Between vagina and urethra: _____ 1 o'clock: _____

Inside vagina: _____ 4-5 o'clock: _____

Posterior fourchette: _____ 6 o'clock: _____

Perineum: _____ 7-8 o'clock: _____

11 o'clock: _____

Appearance of vestibule after cotton-swab test:

No erythema ☐	Erythema ☐	Other ☐ _____

Ask women with provoked pain during sexual activity only:

Does this pain feel like the pain you experience during sexual activity?

Yes No Maybe

If **no**, how was it different? _____

_____

ANAL WINK TEST

Present ☐	Weak ☐	Absent ☐

SECTION III: MUSCLE TENSION ASSESSMENT (random order)

Apply pressure inside the vagina for 3 seconds at 8, 6, and 4 o'clock. Record pain intensity ratings.

8 o'clock: _____ 6 o'clock: _____ 4 o'clock: _____

Evaluation of muscle tension by the examining physician

Relaxed ☐	Tense ☐	Severe tension ☐

Kegel evaluation: degree of contraction

None ☐	Weak ☐	Strong ☐	Very strong ☐

Kegel evaluation: degree of relaxation

None ☐	Relaxed ☐	Tense ☐

SECTION IV: BIMANUAL PALPATION

Palpate the uterus, the adnexae, and the cervix. Describe any and all abnormalities, including pain (fibroids, cysts, endometriosis, etc):

_____

_____

_____

_____

Introitus:

<1 fingerbreadth ☐	1 fingerbreadth ☐	2 fingerbreadths ☐	+2 fingerbreadths ☐

SECTION V: OVERALL UNPLEASANTNESS OF GYNECOLOGICAL EXAMINATION

Ask: On a scale from 0 to 10, how unpleasant overall was the gynecological examination?

_____

SECTION VI: DIAGNOSTIC IMPRESSION

No gynecological diagnosis _____

Vulvar vestibulitis syndrome _____

Generalized vulvodynia _____

Vaginal atrophy _____

Vulvar fissures _____

Lichen planus _____

Lichen sclerosus _____

Heightened muscle tension _____

Other _____ please specify:

_____

_____

Appendix D: Advertisements

PARTICIPATE IN SEX RESEARCH!

Seeking **WOMEN** ages 18-50 who experience genital pain to participate in a study

STUDY PROCEDURES:

- ✓ Brief gynecological exam
- ✓ Interview and questionnaires about sexuality
- ✓ Sensory testing of your hand
- ✓ Two Brain imaging (fMRI) sessions

Participation will take approximately 4 hours (total)
All information is completely confidential

COMPENSATION PROVIDED

INTERESTED?
Visit: Participate.SexLab.ca
Or, for further information, contact:
613.533.3276
Sex.Lab@queensu.ca
Refer to "**fMRI"** Study

sexual health research laboratory

Investigators: Caroline Pukall, Ph.D., Patrick Stroman, Ph.D., Sue Chamberlain, MD, Linda Booij, Ph.D., & Moshe Szyf, Ph.D.

PARTICIPATE IN SEX RESEARCH!

fMRI Study SEX.LAB@queensu.ca

Seeking healthy **WOMEN** ages 18-50 to participate in a study

STUDY PROCEDURES:

- ✓ Interview and questionnaires about sexuality
- ✓ Sensory testing of your hand
- ✓ Two Brain imaging (fMRI) sessions

Participation will take approximately 4 hours (total)

All information is completely confidential

COMPENSATION PROVIDED

INTERESTED?

Visit: Participate.SexLab.ca

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fMRI Study SEX.LAB@queensu.ca

Appendix E: Eligibility Screener

Eligibility screening interview: CCB fMRI study
The following questions will be asked by 1 of 2 methods – whichever best suits the participant:
1. Over the phone or 2. Online

NOTE: the online eligibility screening will be hosted on FluidSurvey. Participants will be given a link to fill out the questionnaire and will only see questions relevant to their group status (control, vulvar pain, or fibromyalgia. Eligibility will be determined by the researcher by reviewing the screening on FluidSurvey, which is password protected and hosted on a secure server. Only Study researchers will have access to this information. Participants will be called to confirm eligibility.

Participant ID # _____

Date of call: _____

1. How did you hear about this study?

1) Poster: Where? _____
2) Online: Which website/social media service? _____
3) Radio ad: Which one? _____
4) Newspaper ad: Which one? _____
5) Word of Mouth
6) Other: How? _____

Study information

**Before beginning ASK THE WOMAN if her pain is genital (opening to vagina) or pelvic (near cervix or cramping) in nature. If it is ONLY pelvic explain that that is not the type of pain that we are studying. (For online screeners, this will appear as a choice between three options including: genital pain/vulvodynia, fibromyalgia, or pain free control. Once selecting a choice, participants will be allowed to proceed to the questions and criteria specific to their group.)

I am just going to tell you a bit about why this research is being done and then explain in detail exactly what your participation entails. Please interrupt if you have questions. (Phone screen only)

The main goal of this study is to determine differences in pain characteristics in women with provoked vestibulodynia (PVD), fibromyalgia, and control women.

PVD, formerly termed vulvar vestibulitis syndrome (VVS) is the most common form of genital pain, affecting about 12% of women in the general population. Despite the prevalence, we still do not know exactly what causes or maintains this pain. It is hoped that the results of this study will provide further information about the underlying causes and of PVD as well as things that might make the pain worse. We are hoping that this study leads to some new possibilities for treatment options. Fibromyalgia is a chronic pain condition that affects more women than men and involves

widespread pain and tenderness, and fatigue. We know very little about what causes and maintains fibromyalgia, and is our hope that we can learn important information about it through this study.

Your participation in this study involves undergoing the following procedures: 1) a telephone screening interview to determine eligibility; 2) a gynecological examination to confirm eligibility [PVD WOMEN ONLY]; 3) online (or hard copy) completion of questionnaires; 4) a sensory testing/sham fMRI session, followed by a saliva collection, and the first functional MRI (fMRI) imaging session; and 5) a second fMRI imaging session. All together this should take approximately 3.5 to 4 hours to complete.

The interview will be conducted online. The interview will cover information such as sociodemographics, medical history, vulvar pain history and pain characteristics (if you suffer from chronic genital pain or fibromyalgia), and sexual and relationship functioning. There will also be questionnaires on a computer asking about sexual and relationship functioning, mental and physical health, personality, sleep, childhood history, and pain (Vulvodynia and fibromyalgia groups). You are under no obligation to answer any questions that you feel uncomfortable answering.

The sensory and fMRI testing will be done by a trained female researcher who conducts the interview and runs the computer and record ratings. FMRI facility staff will be present and may include both males and females. The sensory testing session consists of assessing thermal pain thresholds (e.g., when do you first feel pain) at the hand. During the sensory testing session, a small thermode will be placed on your hand and it will administer different levels of warmth and heat. Since we are interested in seeing how your brain perceive pain, we will choose a level of temperature that is moderately painful for you, for the brain imaging session. We will ask participants to give us a saliva sample by spitting into a sterilized tube.

For all sections of the sensory testing, the researcher will ask you at each time she applies a stimulus to your body if you feel pain or not and will ask you to rate the intensity of the stimuli on scales. Although some pain will be experienced during this examination, no health risks are posed, and the painful sensations do not last for long periods of time. You are able to stop or control the session at any time. For your time and inconvenience, you will be reimbursed [$75.00 (Vulvar pain group); $60 (fibro and control groups)] upon completion of all parts of the study.

Do you have any questions? Answer any questions the participant may have. (Phone screen only)

Are you interested in seeing if you are eligible for participating in the study?
(All phone screening scripts will be displayed as popup messages for online participants)

YES → Thank them and ask for their full name: _____

NO → Thank them for their time, and ask them to feel free to call back if they change their mind. End the screening interview.

Do you consent to allowing the Sexual Health Research Lab to save your screening information

in our secure database? This means that the personal information we gather (e.g., age, name, phone, email, etc.) is kept in one database file and the screening questionnaire answer are kept in another database file. These two databases are NOT directly linked. Should you be eligible, we would link your MASTER ID with your unique participant study ID in the secure database. Only the lead investigator and research delegate can access the study-specific database, which does not contain any identifying information.

YES – continue
NO – if no, do not link master ID to study screening and delete record if not eligible.

1. How old are you? _____ * must be between the ages of 18 and 50

2. Are you right or left handed? _____ *Still eligible if left handed

3. Are you currently using hormonal contraceptives? YES NO

If YES,

2a) What type? _____ * if IUD they are not eligible due to FMRI

2b) For how long? _____

Do you mind answering some questions about your medical history to determine if you are eligible for the study?

If YES → Explain that we need to ask these questions to determine their eligibility for the study. If they are still hesitant, ask them to think about it and call back.

4. Are you currently suffering from any medical or psychiatric conditions?

YES* NO → go to #6

→ If **yes**, what condition(s) have you been diagnosed with?

_____

→ Are you taking any medications for this/these conditions? YES* NO

→ If **yes**, which one(s)?

_____

→ Are you receiving any other treatment for this/these conditions? YES* NO

→ If **yes**, which one(s)?

_____

→ Have you previously taken any medication or received any other treatment for this/these conditions?
YES* NO

→ If **yes**, which one(s)?

_____

5. Have you ever suffered, or are you currently suffering, from a chronic or recurrent pain condition (other than genital pain)? Note: Chronic pain refers to persistent pain that often lasts for months (e.g., back pain). Recurrent pain refers to pain episodes that reoccur that are interspersed with pain-free episodes (e.g., migraine headaches). *If They have Fibromyalgia, continue screening and be sure to ask group specific questions highlighted below (#18-19).

YES* NO → go to #6

→ If **yes**, what condition(s) have you been diagnosed with?

_____

→ When did this episode/these episodes occur?

_____

→ How long did this episode/these episodes last?

_____

→ Are you currently taking painkillers for this/these conditions? YES* NO → go to #7

→ If **yes**, which one(s)?

_____

6. When was your last gynecological examination?

_____

→ Was it painful? YES NO

7. Do you use tampons or any other kind of internal feminine hygiene product (e.g., Keeper/Diva Cup)?

YES NO

7b) Do you experience pain when you insert/remove tampons/product? YES NO

8. Have you ever given birth? YES NO → go to #9

→ If **YES**: How many children do you have? _____

→ Through what method/s of delivery?

A) Vaginal delivery: How many? _____

B) Caesarean-section: How many? _____

→ Are you currently breast-feeding? YES* NO

If YES, thank the woman for her time, but she is not eligible. Remind her that she is welcome to contact the lab for future studies or to complete the online study.

9. Is there any possibility that you might currently be pregnant? YES* NO

If YES, thank the woman for her time, but she is not eligible. Remind her that she is welcome to contact the lab for future studies or to complete the online study.

10. What was the start date of your last menstrual period? _____

11. Do you have any difficulty at all with vaginal penetration or insertion? YES* NO
→ go to #14

→ If **yes**, please describe:

_____

12. Do you currently experience pain in your genital region? YES NO

YES: For how long have you had this pain? _____ (min 6 months)
→ Go to #13 and use present tense

NO: Have you **ever** had recurrent and persistent genital pain?
→ YES: Why do you no longer have the pain?

_____
Go to #13 and use past tense

→ NO skip ahead to #18

VULVAR PAIN WOMEN ONLY **(Questions 13 to 17)**

13. In what situations do/did you feel the pain?

A) It is always or almost always present

B) During sexual intercourse or activities involving vaginal penetration: Which activities?

C) It is always or almost always there and worsens during sexual intercourse/activities involving vaginal penetration: Which activities?

_____

D) Other:

_____

If B or C is endorsed: **When does/did the pain START (or worsen) during sexual intercourse or activities involving vaginal penetration?**

A) When the penis/finger/object starts to enter the vagina

B) When the penis/finger/object has fully entered and is thrusting*

C) Only after penetration: How long does it last? _____

14. In which genital areas do/did you feel the pain?

A) At the vaginal opening

B) Everywhere on the vulva

C) Inside the vagina

D) In the pelvic or abdominal region*

E) Other:

_____

15. What adjective/s would you use to describe the pain you feel in your genital region?

---

16. Did you receive any diagnosis for this pain? YES NO → go to #17

→ If **yes**, what diagnosis/diagnoses did you receive?

_____

→ By whom?

_____

→ When?

_____

17. Have you ever undergone any treatment for the pain? YES NO → go to #18

→ If **yes**, which one(s)?

_____

→ Are you currently undergoing any treatment? YES NO → go to #13

→ If yes, which one/s?

_____

FIBROMYALGIA WOMEN ONLY

18. Please provide details about your fibromyalgia, such how long ago were you diagnosed, what symptoms do you have, etc.

19. Do you have any altered pain sensitivity? If yes, please provide detailed information (i.e. what type of pain or sensitivity? which part(s) of your body? severity? etc?)

TO ALL PARTICIPANTS – MRI QUESTIONS

20. To determine whether you are eligible for the FMRI testing I am going to read out the FMRI safety checklist. Please indicate whether or not you have any of the following.

	YES	NO
Aneurysm clips (brain, abdominal, carotid)		
Cardiac pacemaker, internal electrodes or wires		
Internal defibrillator		
Prosthetic heart valve replacement		
Electronic implant or device		
Magnetically activated implant or device		
Occupational history as a sheet metal worker, machinist, welder, etc		
History of metal fragments in the eye, head or body		
Shrapnel or gunshot wound		
Orbital/eye prosthesis, eyelid spring or wire		
Cochlear, otologic or other ear implant		
Any type of intravascular coil, filter or stent		
Shunt (spinal or intraventricular)		
Swan-Ganz or thermo dilution catheter		
Implanted neurostimulator (spinal cord, bone, etc)		
Vascular access port and/or catheter		
Implanted drug infusion device (insulin, etc)		
Tattoo or permanent eyeliner		
Body piercing * (removable is fine) _____		
Radiation seeds or implants		
Artificial or prosthetic limb		

Joint replacement (hip, knee, etc)		
Bone or joint pin, screw, nail, wire, plate, etc		
Surgical staples, clips, metallic structures or wire mesh		
Other implant _____		
Breathing problem or motion disorder		
Dentures or partial plates		
Hearing aid		
Medication patch		
Claustrophobia		
Are you pregnant or breast feeding?		
Breast implants or tissue expanders		
IUD, diaphragm or pessary		

*** If YES to any of the items on the list they may not be eligible for the study. Please tell them that you need to consult with your supervisor and will get back to them.**
***EXCEPTION is removable body piercings.**

21. If you have ever had a spinal cord injury, please provide detailed information (i.e. how long ago? what level of your spine? cause of injury? severity of injury? (If yes, not eligible)

22. If you have ever had a brain injury, please provide detailed information (i.e. how long ago? cause of injury? type of injury? effects of injury? etc?) (If yes, not eligible)

23. Have you had prior surgery or an operation of any kind?

24. Do you have any history of heart disease (angina, palpitations, heat attack, etc.)?

FOR ONLINE ELIGIBILITY SCREENING
Last page of questionnaire will ask for a phone number and best time to reach them to confirm eligibility.

Initial Decision

NOT ELIGIBLE* _____ (If they are not eligible, explain to them why and thank them for their time. Suggest that they might be interested in another study.)

NOT SURE ____ (If not sure, tell them that you will call them back after discussing it with your supervisor.)

CONTROL GROUP _____† (Tell them that they are eligible for the study.)

PVD _____† (Tell them that they are eligible for this study.)

FIBRO_____

†**Are you interested in participating in the study?** YES NO NOT SURE

→ If **no/not sure**, thank them for their time and ask them to call back if they change their minds. Answer any questions they might have, especially if they are not sure.

→ If **yes**, ask: Book the gynecological exam according to Sue's schedule.

Gynecological Exam Date/time booked:
_____

→ What is the best way to contact you in order to confirm the appointment?
NOTE: Inform them that if we leave a message, we would leave only our name and number and that we are calling from Queen's University.

Home: _____ Can we leave you a message? YES NO

Work: _____ Can we leave you a message? YES NO

Cell: _____ Can we leave you a message? YES NO

Email address: _____

Appendix F: fMRI Protocol

Spinal/Brain fMRI : Studies of Descending Modulation of Pain

Behavioral Testing and Calibration

Name:_____ Subject I.D. _____
Date:_____

1. Have participants fill in anxiety questionnaire.
    a. *The first thing we will have you do is fill in several questionnaires. These questionnaires ask questions about anxiety and pain catastrophizing. They are for control purposes only and all answers will be confidential. Throughout the study we will be monitoring your feelings of anxiety using this anxiety scale. The scale is from 0-10, where 0 means you feel calm and at ease and 10 means you feel as if you may have an anxiety attack.*
    b. *Please provide a rating of the anxiety that you are experiencing right now- using this scale.*
    c. *We will be asking you to rate your anxiety as you just did throughout the study. If at any point you need to be reminded of the scale anchors, let me know.*
2. Discuss the purpose of the study
    a. *The purpose of this study is … (e.g. to see how your spinal cord process heat pain)*
    b. *Everyone perceives pain differently, and we are interested in learning about how women with PVD/fibromyalgia perceive pain differently from those who do not have PVD/fibromyalgia. This study is about learning about how you perceive pain, it is not a tolerance study. We are not asking you to tell us how much pain you can tolerate, but instead give an accurate rating about the pain that you are experiencing.*
3. Describe pain scales
    a. *This is the scale that we will use for you to describe your pain experience to the heat pain that we are going to administer. This scale is from 0 – 100. 0 means that you do not feel anything, while a rating of 10 means that the sensation is warm. A rating of 20 indicates that the temperature is considered painful. So if you experience pain, you should rate your experience a 20 or higher. A rating of 100 is considered intolerable. If you are rating it in this range, it would make you want to pull the thermode off your hand quickly, because the pain cannot be tolerated. We will not be using temperatures in this range for this study.*
    b. *Please provide a rating of your overall pain intensity that you are experiencing right now- using this scale*

Pain Intensity:_____ Time:_____

4. Threshold testing
    a. *For the next parts of the study, we will be using the pain intensity scale to rate the pain you experience.*
    b. *In order to study your pain perception, you will experience pain via a thermal stimulation device (so heat pain), however this pain will be calibrated for your own pain threshold and will not be intolerable. This is called a thermode, it provides the heat stimuli. We will now place the thermode on your right hand and we will have you rate your pain using the pain intensity scale. You should position your hand so the thermode will touch the fleshiest part of the heel of your thumb (indicate where on your own hand).*
    c. *Using the pain intensity scale (remind them of the scale) please rate your pain*
        i. **If participants rate their pain over a "70" at any time, ask them to remove their hand and remind them that we do not expect them to tolerate temperatures that are intolerable.*

Ascending Temps:
Temps: 45-45-45 1:_____ 2:_____ 3: _____
Temps: 47-47-47 1:_____ 2:_____ 3: _____
Temps: 46-46-46 1:_____ 2:_____ 3: _____

5. Rating each pulse, and testing
    a. *Now we will begin the actual test and like before you will indicate your rating to each pulse. However, this time instead of three pulses you will feel 10 pulses in a row*
    b. *After the pulses are done, we ask that you do not rub your hand, but instead that you focus on the after sensations. I will also have you rate what you feel 15 and 30 seconds after the last pulse.*
        i. **Do not start the next set until the sensations are back to 10 or below*

Temperature (46, 50, 44, 48)				
Pulse 1				
Pulse 2				
Pulse 3				
Pulse 4				
Pulse 5				
Pulse 6				
Pulse 7				
Pulse 8				
Pulse 9				
Pulse 10				
AS 15 s				
AS 30 s				

(must ensure participant is back to baseline before starting the next one)

6. Sham
    a. *We will now continue by having you experience the sham and practice making ratings to your pain sensation while you are in the MRI-like environment, laying down and listening to the noises. Again we will have you think about your ratings to each pulse.*
    b. *We want you to pay attention to each pulse., however, because the MRI is sensitive to movement, we do not want you to say your rating to each pulse because talking requires moving. Instead, we want you to rate the first pulse in your mind, remember your rating, and then report your rating to the first and to the last pulse after the imaging is done.*
    c. *It is really important that you think of your rating as you feel the pulses of heat as ratings change when you think back to your pain experience compared to reporting what you feel in real time.*

Type	*Temperature*	*First pain rating*	*Last pain rating*	*Description*	*Notes*

7. Final ratings
    a. *Finally, I will again get your overall pain intensity rating*

PI:_____ Time:_____

FMRI Session

fMRI acquisition protocol:
HASTE, 280 mm x 210 mm FOV, sagittal slices, 2 mm slice thickness, 0 gap between slices, 9 slices, 192 x 144 matrix, acquire 84 phase encoding steps (partial Fourier with ½ of k-space + 12 lines of oversampling), pixel size 1.46 mm x 1.46 mm, TE = 76 msec, TR = 750 msec per slice, Rx bandwidth = 790 Hz/pixel
No oversampling in phase encoding direction, Flow compensation in H/F direction, spatial saturation pulses to eliminate signal anterior to the spine

Repeat the acquisition 40 times to produce a 4 minute 30 second time series.

1) 3-plane localizer, 30 cm FOV

2) coronal localizer, 30 cm FOV

3) Sagittal HASTE – one volume, same parameters as what will be used for fMRI – to check position

Runs 4 ...

4, 5, ...) Spinal fMRI using one of the patterns in the table below

When scan starts – start visual display and audio cues program ___ 40 time points

1 minute baseline, tell participant study type (resting or stim), 10 contact heat pulses starting at 2:00 and ending at 2:30, rest period ending at 4:30

Series number	Pattern 1	Pattern 2	Pattern 3
4	stim	stim	stim
5	stim	rest	stim
6	rest	rest	stim
7	stim	stim	rest
8	rest	rest	stim
9	rest	stim	rest
10	stim	stim	rest
11	stim	rest	stim
12	rest	rest	rest
13	rest	stim	rest

Record pain ratings for each run:

run number	Set temp.	Run start time	run type	1st contact rating	last contact rating	Series Number
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Finish time: _____

Appendix G: Validated Measures

***Note these questionnaires will be programmed into FluidSurveys and will always have a decline response option**

Only certain questionnaires will be administered based on the group: vulvodynia (PVD and GVD) or control.

Female Sexual Function Index (FSFI) (All Groups)

INSTRUCTIONS: These questions ask about your sexual feelings and responses during the past 4 weeks. Please answer the following questions as honestly and clearly as possible. Your responses will be kept completely confidential. In answering these questions the following definitions apply:

Sex, **sexual activity**, and **foreplay** refer to:
– caressing, kissing, manual stimulation of the genitals/anus/breasts by yourself or your partner
– oral stimulation of the genitals/anus/breasts
– vaginal or anal penetration with penis, fingers, or sex toys

Sexual intercourse refers to:
– penetration of your partner's vagina/anus with fingers or sex toys
– receiving vaginal/anal penetration with penis, fingers, or sex toys

Sexual stimulation refers to sexual situations such as the following:
– foreplay with your partner, stimulating your partner, receiving stimulation from your partner,
– self-stimulation (masturbation), sexual fantasy
– viewing erotic films, pictures, or reading erotic material

CHECK ONLY ONE BOX PER QUESTION.

Sexual desire or interest is a feeling that includes wanting to have a sexual experience, feeling receptive to a partner's sexual initiation, and thinking or fantasizing about having sex.

1. Over the past 4 weeks, how **often** did you feel sexual desire or interest?

☐ Almost always or always
☐ Most times (more than half the time)
☐ Sometimes (about half the time)
☐ A few times (less than half the time)
☐ Almost never or never

2. Over the past 4 weeks, how would you rate your **level** (degree) of sexual desire or interest?

☐ Very high
☐ High
☐ Moderate

☐ Low
☐ Very low or none at all

Sexual arousal is a feeling that includes both physical and mental aspects of sexual excitement. It may include feelings of warmth or tingling in the genitals, lubrication (wetness), or muscle contractions.

3. Over the past 4 weeks, how **often** did you feel sexually aroused ("turned on") during sexual activity or intercourse?
☐ No sexual activity
☐ Almost always or always
☐ Most times (more than half the time)
☐ Sometimes (about half the time)
☐ A few times (less than half the time)
☐ Almost never or never

4. Over the past 4 weeks, how would you rate your **level** of sexual arousal ("turn on") during sexual activity or intercourse?
☐ No sexual activity
☐ Very high
☐ High
☐ Moderate
☐ Low
☐ Very low or none at all

5. Over the past 4 weeks, how **confident** were you about becoming sexually aroused during sexual activity or intercourse?
☐ No sexual activity
☐ Very high confidence
☐ High confidence
☐ Moderate confidence
☐ Low confidence
☐ Very low or no confidence

6. Over the past 4 weeks, how **often** have you been satisfied with your arousal (excitement) during sexual activity or intercourse?
☐ No sexual activity
☐ Almost always or always
☐ Most times (more than half the time)
☐ Sometimes (about half the time)
☐ A few times (less than half the time)
☐ Almost never or never

7. Over the past 4 weeks, how **often** did you become lubricated ("wet") during sexual activity or intercourse?

☐ No sexual activity
☐ Almost always or always
☐ Most times (more than half the time)
☐ Sometimes (about half the time)
☐ A few times (less than half the time)
☐ Almost never or never

8. Over the past 4 weeks, how **difficult** was it to become lubricated ("wet") during sexual activity or intercourse?
☐ No sexual activity
☐ Extremely difficulty or impossible
☐ Very difficult
☐ Difficult
☐ Slightly difficult
☐ Not difficult

9. Over the past 4 weeks, how often did you **maintain** your lubrication ("wetness") until completion of sexual activity or intercourse?
☐ No sexual activity
☐ Almost always or always
☐ Most times (more than half the time)
☐ Sometimes (about half the time)
☐ A few times (less than half the time)
☐ Almost never or never

10. Over the past 4 weeks, how **difficult** was it to maintain your lubrication ("wetness") until completion of sexual activity or intercourse?
☐ No sexual activity
☐ Extremely difficulty or impossible
☐ Very difficult
☐ Difficult
☐ Slightly difficult
☐ Not difficult

11. Over the past 4 weeks, when you had sexual stimulation or intercourse, how **often** did you reach orgasm (climax)?
☐ No sexual activity
☐ Almost always or always
☐ Most times (more than half the time)
☐ Sometimes (about half the time)
☐ A few times (less than half the time)
☐ Almost never or never

12. Over the past 4 weeks, when you had sexual stimulation or intercourse, how **difficult** was it for you to reach orgasm (climax)?
☐ No sexual activity

- ☐ Extremely difficulty or impossible
- ☐ Very difficult
- ☐ Difficult
- ☐ Slightly difficult
- ☐ Not difficult

13. Over the past 4 weeks, how **satisfied** were you with your ability to reach orgasm (climax) during sexual activity or intercourse?

- ☐ No sexual activity
- ☐ Very satisfied
- ☐ Moderately satisfied
- ☐ About equally satisfied and dissatisfied
- ☐ Moderately dissatisfied
- ☐ Very dissatisfied

14. Over the past 4 weeks, how **satisfied** have you been with the amount of emotional closeness during sexual activity between you and your partner?

- ☐ No sexual activity
- ☐ Very satisfied
- ☐ Moderately satisfied
- ☐ About equally satisfied and dissatisfied
- ☐ Moderately dissatisfied
- ☐ Very dissatisfied

15. Over the past 4 weeks, how **satisfied** have you been with your sexual relationship with your partner?

- ☐ Very satisfied
- ☐ Moderately satisfied
- ☐ About equally satisfied and dissatisfied
- ☐ Moderately dissatisfied
- ☐ Very dissatisfied

16. Over the past 4 weeks, how **satisfied** have you been with your overall sexual life?

- ☐ Very satisfied
- ☐ Moderately satisfied
- ☐ About equally satisfied and dissatisfied
- ☐ Moderately dissatisfied
- ☐ Very dissatisfied

17. Over the past 4 weeks, how **often** did you experience discomfort or pain <u>during</u> vaginal penetration?

- ☐ Did not attempt intercourse
- ☐ Almost always or always
- ☐ Most times (more than half the time)
- ☐ Sometimes (about half the time)
- ☐ A few times (less than half the time)

- ☐ Almost never or never

18. Over the past 4 weeks, how **often** did you experience discomfort or pain <u>following</u> vaginal penetration?

- ☐ Did not attempt intercourse
- ☐ Almost always or always
- ☐ Most times (more than half the time)
- ☐ Sometimes (about half the time)
- ☐ A few times (less than half the time)
- ☐ Almost never or never

19. Over the past 4 weeks, how would you rate your **level** (degree) of discomfort or pain during or following vaginal penetration?

- ☐ Did not attempt intercourse
- ☐ Very high
- ☐ High
- ☐ Moderate
- ☐ Low
- ☐ Very low or none at all

<u>Beck Depression Inventory (BDI) (All Groups)</u>

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. **Sadness**

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all of the time.
3 I am so sad or unhappy that I can't stand it.

2. **Pessimism**

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. **Past Failure**

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel like I am a total failure as a person.

4. **Loss of Pleasure**

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. **Guilty Feelings**

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. **Punishment Feelings**

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. **Self-Dislike**

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. **Self-Criticalness**

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. **Suicidal Thoughts or Wishes**

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.

2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. **Crying**

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

11. **Agitation**

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. **Loss of Interest**

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. **Indecisiveness**

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. **Worthlessness**

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. **Loss of Energy**

0 I have as much energy as ever.
1 I have less energy than I used to have.

2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. **Changes in Sleeping Pattern**

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1-2 hours early and can't get back to sleep.

17. **Irritability**

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. **Changes in Appetite**

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. **Concentration Difficulty**

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. **Tiredness or Fatigue**

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to.
3 I am too tired or fatigued to do most of the things I used to do.

21. **Loss of Interest in Sex**

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

State Trait Anxiety Inventory-State (STAI-S) (ALL Groups)

Directions: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number above the statement to indicate how you feel ***right*** now, that is, ***at this moment***. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

1: Not at all
2: Somewhat
3: Moderately so
4: Very much so

1. I feel calm	1	2	3	4	
2. I feel secure		1	2	3	4
3. I am tense	1	2	3	4	
4. I feel strained		1	2	3	4
5. I feel at ease		1	2	3	4
6. I feel upset	1	2	3	4	
7. I am presently worrying over possible misfortunes			1	2	3
4					
8. I feel satisfied		1	2	3	4
9. I feel frightened	1	2	3	4	
10. I feel comfortable	1	2	3	4	
11. I feel self-confident		1	2	3	4
12. I feel nervous	1	2	3	4	
13. I am jittery		1	2	3	4

14. I feel indecisive	1	2	3	4	
15. I am relaxed		1	2	3	4
16. I feel content		1	2	3	4
17. I am worried		1	2	3	4
18. I feel confused	1	2	3	4	
19. I feel steady		1	2	3	4
20. I feel pleasant	1	2	3	4	

State Trait Anxiety Inventory- Trait (STAI-T) (ALL Groups)

Directions: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number above the statement to indicate how you ***generally feel***. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you ***generally feel***.

1: Almost never
2: Sometimes
3: Often
4: Almost always

1. I feel pleasant		1	2	3	4
2. I feel nervous and restless	1	2	3	4	
3. I feel satisfied with myself	1	2	3	4	
4. I wish I could be as happy as others seem to be		1	2	3	4
5. I feel like a failure	1	2	3	4	
6. I feel rested	1	2	3	4	
7. I am "calm, cool, and collected"		1	2	3	4
8. I feel that difficulties are piling up so that I cannot overcome them			1	2	3 4
9. I worry too much over something that really doesn't matter			1	2	3 4
10. I am happy	1	2	3	4	
11. I have disturbing thoughts		1	2	3	4
12. I lack self-confidence		1	2	3	4
13. I feel secure		1	2	3	4
14. I make decisions easily	1	2	3	4	
15. I feel inadequate	1	2	3	4	
16. I am content		1	2	3	4

17. Some unimportant thought runs through my mind and bothers me 4	1	2	3	
18. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

The SF-36 is a 36 item survey measuring health-related quality-of-life. The SF-36 assesses eight health domains: limitations in physical activities due to limitations in social activities because of physical or emotional problems, limitations in usual role activities because of physical health problems, bodily pain, general mental health (psychological distress and well-being), limitations in usual role activities because of emotional problems, vitality (energy and fatigue), and general health perceptions Ware & Sherbourn, 1992). Participants are asked to rate their responses on three or six point scales. Scores are coded, summed, and then converted to a 0 (poor health)-100 (good health) scale. There is considerable reliability of the SF-36 (Cronbach's a >0-85, reliability coefficient >0 75 for all dimensions except social functioning) (Brazier et al., 1992). The SF-36 is able to detect low levels of poor health (Brazier et al., 1992).

SF-36 (Hayes et al., 1993) (ALL GROUPS)

---

1. In general, would you say your health is:
    a. Excellent
    b. Very good
    c. Good
    d. Fair
    e. Poor

2. Compared to one year ago, how would you rate your health in general now?
    a. Much better now than one year ago
    b. Somewhat better now than one year ago
    c. About the same
    d. Somewhat worse now than one year ago
    e. Much worse now than one year ago

The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? (Questions 3-12)
    a. Yes, limited a lot
    b. Yes, limited a little
    c. No, not limited at all

3. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
4. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
5. Lifting or carrying groceries
6. Climbing several flights of stairs
7. Climbing one flight of stairs
8. Bending, kneeling, or stooping
9. Walking more than a mile
10. Walking several blocks
11. Walking one block
12. Bathing or dressing yourself

During the past *4 weeks*, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? (Questions 13-16)

a. Yes
b. No

13. Cut down the amount of time you spent on work or other activities
14. Accomplished less than you would like
15. Were limited in the kind of work or other activities
16. Had difficulty performing the work or other activities (for example, it took extra effort)

During the past *4 weeks*, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)? (Questions 17-19)

a. Yes
b. No

17. Cut down the amount of time you spent on work or other activities
18. Accomplished less than you would like
19. Didn't do work or other activities as carefully as usual

20. During the past *4 weeks*, to what extent have your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

a. Not at all
b. Slightly
c. Moderately
d. Extremely
e. Quite a bit

21. How much bodily pain have you had during the past *4 weeks*?

a. None
b. Very mild
c. Mild
d. Moderate
e. Severe
f. Very severe

22. During the past *4 weeks*, how much did pain interfere with your normal work (including both work outside the home and housework)?

a. Not at all
b. A little bit
c. Moderately
d. Quite a bit
e. Extremely

These questions are about how you feel and how things have been with you during the past *4 weeks*. For each question, please give the one answer that comes closest to the way you have been feeling. (Questions 23-31)

a. All of the time
b. Most of the time
c. A good bit of the time
d. Some of the time
e. A little of the time
f. None of the time

How much of the time during the past *4 weeks*...

23. Did you feel full of pep?
24. Have you been a very nervous person?
25. Have you felt so down in the dumps that nothing could cheer you up?
26. Have you felt calm and peaceful?
27. Did you have a lot of energy?
28. Have you felt downhearted and blue?
29. Did you feel worn out?
30. Have you been a happy person?
31. Did you feel tired?

32. During the past *4 weeks*, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

a. All of the time
b. Most of the time
c. A good bit of the time
d. Some of the time
e. A little of the time
f. None of the time

How TRUE or FALSE is each of the following statements for you. (Questions 33-36)

a. Definitely true
b. Mostly true
c. Don't know
d. Mostly false
e. Definitely false

33. I seem to get sick a little easier than other people
34. I am as healthy as anybody I know
35. I expect my health to get worse
36. My health is excellent

Big Five Inventory (ALL GROUPS)

(John & Srivastava, 1999)

John, O., & Srivastava, S. (1999). The big-five trait taxonomy history, measurement, and

theoretical perspectives. *In Handbook of personality: Theory and research* (2nd ed.). New York: Guilford.

Here are a number of characteristics that may or may not apply to you. For example, do you agree that you are someone who likes to spend time with others? Please write a number next to each statement to indicate the extent to which you agree or disagree with that statement.

1	2	3	4	5
Disagree strongly	Disagree a little	Neither agree nor disagree	Agree a little	Agree strongly

I see Myself as Someone Who...

_____1. Is talkative
____2. Tends to find fault with others
____3. Does a thorough job
____4. Is depressed, blue
____5. Is original, comes up with new ideas
____6. Is reserved
____7. Is helpful and unselfish with others
____8. Can be somewhat careless
____9. Is relaxed, handles stress well
____10. Is curious about many differentthings
____11. Is full of energy
____12. Starts quarrels with others
____13. Is a reliable worker
____14. Can be tense
____15. Is ingenious, a deep thinker
____16. Generates a lot of enthusiasm
____17. Has a forgiving nature
____18. Tends to be disorganized
____19. Worries a lot
____20. Has an active imagination
____21. Tends to be quiet
____22. Is generally trusting
____23. Tends to be lazy
____24. Is emotionally stable, not easily upset
____25. Is inventive
____26. Has an assertive personality
____27. Can be cold and aloof
____28. Perseveres until the task is finished
____29. Can be moody
____30. Values artistic, aesthetic experiences
____31. Is sometimes shy, inhibited
____32. Is considerate and kind to almost everyone
____33. Does things efficiently
____34. Remains calm in tense situations
____35. Prefers work that is routine
____36. Is outgoing, sociable
____37. Is sometimes rude to others
____38. Makes plans and follows through with them
____39. Gets nervous easily
____40. Likes to reflect, play with ideas
____41. Has few artistic interests
____42. Likes to cooperate with others
____43. Is easily distracted
____44. Is sophisticated in art, music, or literature

BIDR Version 6 - Form 40A (ALL GROUPS)
Balanced Inventory of Desirable Responding (Paulhus, 1984, 1988)

Using the scale below as a guide, write a number beside each statement to indicate how true it is.

```
+_____+_____+_____+_____+_____+_____+
1     2     3     4     5     6     7
not true          somewhat          very true
```

____ 1. My first impressions of people usually turn out to be right.

____ 2. It would be hard for me to break any of my bad habits.

____ 3. I don't care to know what other people really think of me.

____ 4. I have not always been honest with myself.

____ 5. I always know why I like things.

____ 6. When my emotions are aroused, it biases my thinking.

____ 7. Once I've made up my mind, other people can seldom change my opinion.

____ 8. I am not a safe driver when I exceed the speed limit.

____ 9. I am fully in control of my own fate.

____ 10. It's hard for me to shut off a disturbing thought.

____ 11. I never regret my decisions.

____ 12. I sometimes lose out on things because I can't make up my mind soon enough.

____ 13. The reason I vote is because my vote can make a difference.

____ 14. My parents were not always fair when they punished me.

____ 15. I am a completely rational person.

____ 16. I rarely appreciate criticism.

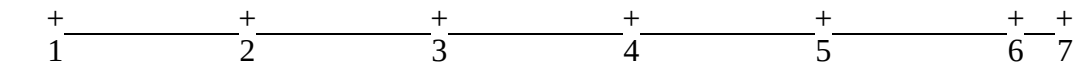
____ 17. I am very confident of my judgments

____ 18. I have sometimes doubted my ability as a lover.

____ 19. It's all right with me if some people happen to dislike me.

____ 20. I don't always know the reasons why I do the things I do.

Using the scale below as a guide, write a number beside each statement to indicate how true it is.



____ 21. I sometimes tell lies if I have to.

____ 22. I never cover up my mistakes.

____ 23. There have been occasions when I have taken advantage of someone.

____ 24. I never swear.

____ 25. I sometimes try to get even rather than forgive and forget.

____ 26. I always obey laws, even if I'm unlikely to get caught.

____ 27. I have said something bad about a friend behind his/her back.

____ 28. When I hear people talking privately, I avoid listening.

____ 29. I have received too much change from a salesperson without telling him or her.

____ 30. I always declare everything at customs.

____ 31. When I was young I sometimes stole things.

____ 32. I have never dropped litter on the street.

____ 33. I sometimes drive faster than the speed limit.

____ 34. I never read sexy books or magazines.

____ 35. I have done things that I don't tell other people about.

____ 36. I never take things that don't belong to me.

____ 37. I have taken sick-leave from work or school even though I wasn't really sick.

____ 38. I have never damaged a library book or store merchandise without reporting it.

____ 39. I have some pretty awful habits.

____ 40. I don't gossip about other people's business.

MOS sleep scale (MOS-S; Hays & Stewart, 1992). (ALL GROUPS)
This scale measures sleep
disturbances. Two items assess how long it takes to fall asleep and how much sleep one gets per night. An additional 10 items rated on a scale from 1 (All of the Time) to 6 (None of the Time) assess the frequency of various sleep problems (e.g., having trouble falling asleep, feeling drowsy during the day). Nine of the items are averaged to create the Sleep Problem Index (Spitzer & Hays, 2003). Total sleep disturbance scores range from 0 to 100. This set of questions has been shown to discriminate between those with and without chronic pain, and is reliable (!s > .63; Hays, Martin, Sesti, & Spritzer, 2005).

Sleep Scale from the Medical Outcomes Study

1. How long did it usually take for you to fall asleep during the past 4 weeks?

(Circle One)

0-15 minutes……..…1

16-30 minutes……..2

31-45 minutes……..3

46-60 minutes……..4

More than 60 minutes …….5

---

2. On the average, how many hours did you sleep each night during the past 4 weeks?

Write in number
of hours per night: ☐☐

How often during the past 4 weeks did you…

(Circle One Number On Each Line)

	All of the Time ▼	Most of the Time ▼	A Good Bit of the Time ▼	Some of the Time ▼	A Little of the Time ▼	None of the Time ▼
3. feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, etc., while sleeping)?	1	2	3	4	5	6
4. get enough sleep to feel rested upon waking in the morning?	1	2	3	4	5	6
5. awaken short of breath or with a headache?	1	2	3	4	5	6
6. feel drowsy or sleepy during the day?	1	2	3	4	5	6
7. have trouble falling asleep?	1	2	3	4	5	6
8. awaken during your sleep time and have trouble falling asleep again?	1	2	3	4	5	6
9. have trouble staying awake during the day?	1	2	3	4	5	6
10. snore during your sleep?	1	2	3	4	5	6
11. take naps (5 minutes or longer) during the day?	1	2	3	4	5	6
12. get the amount of sleep you needed?	1	2	3	4	5	6

Hays, R. D., & Stewart, A. L. (1992). Sleep measures. In A. L. Stewart & J. E. Ware (eds.), Measuring functioning and well-being: The Medical Outcomes Study approach (pp. 235-259), Durham, NC: Duke University Press.

Childhood Traumatic Events Scale Pennebaker, J.W. & Susman, J.R. . (2013) (ALL GROUPS)

For the following questions, answer each item that is relevant. Be as honest as you can. Each question refers to any event that you may have experienced
prior to the age of 17

1. Prior to the age of 17, did you experience a death of a very close friend or family member?_____ If yes, how old were you?_____
    a. If yes, how traumatic was this? (using a 7-point scale, where 1 = not at all traumatic, 4 = somewhat traumatic, 7 = extremely traumatic)_____
    b. If yes, how much did you confide in others about this traumatic experience at the time? (1 = not at all, 7 = a great deal)_____
2. Prior to the age of 17, was there a major upheaval between your parents (such as divorce, separation)?_____ If yes, how old were you?_____
    a. If yes, how traumatic was this? (where 7 = extremely traumatic)_____
    b. If yes, how much did you confide in others? (7 = a great deal)_____
3. Prior to the age of 17, did you have a traumatic sexual experience (raped, molested, etc.)?_____ If yes, how old were you?_____
    a. If yes, how traumatic was this? (7 = extremely traumatic)_____
    b. If yes, how much did you confide in others? (7 = a great deal)_____
4. Prior to the age of 17, were you the victim of violence (child abuse, mugged or assaulted --other than sexual)?_____ If yes, how old were you?_____
    a. If yes, how traumatic was this? (7 = extremely traumatic)_____
    b. If yes, how much did you confide in others? (7 = a great deal)_____
5. Prior to the age of 17, were you extremely ill or injured?_____ If yes, how old were you?_____
    a. If yes, how traumatic was this? (7 = extremely traumatic)_____
    b. If yes, how much did you confide in others? (7 = a great deal)_____
6. Prior to the age of 17, did you experience any other major upheaval that you think may have shaped your life or personality significantly?_____
    a. If yes, how old were you?_____
    b. If yes, what was the event?_____
    c. If yes, how traumatic was this? (7 = extremely traumatic)_____
    d. If yes, how much did you confide in others? (7 = a great deal)_____

Recent Traumatic Events Scale

For the following questions, again answer each item that is relevant and again be as honest as you can. Each question refers to any event that you may have experienced within the last 3 years

1. Within the last 3 years, did you experience a death of a very close friend or family member?_____

    a. If yes, how traumatic was this? (1 = not at all traumatic, 7 = extremely traumatic)_____
    b. If yes, how much did you confide in others about the experience at the time? (1 = not at all, 7 = a great deal)_____
2. Within the last 3 years, was there a major upheaval between you and your spouse (such as divorce, separation)?_____
    a. If yes, how traumatic was this?_____
    b. If yes, how much did you confide in others?_____
3. Within the last 3 years, did you have a traumatic sexual experience (raped, molested, etc.)?_____
    a. If yes, how traumatic was this?_____
    b. If yes, how much did you confide in others?_____
4. Within the last 3 years, were you the victim of violence (other than sexual)?_____
    a. If yes, how traumatic was this?_____
    b. If yes, how much did you confide in others?_____
5. Within the last 3 years, were you extremely ill or injured?_____
    a. If yes, how traumatic was this?_____
    b. If yes, how much did you confide in others?_____
6. Within the last 3 years, has there been a major change in the kind of work you do (e.g., a new job, promotion, demotion, lateral transfer)?_____
    a. If yes, how traumatic was this?_____
    b. If yes, how much did you confide in others?_____
7. Within the last 3 years, did you experience any other major upheaval that you think may have shaped your life or personality significantly?_____
    a. If yes, what was the event?_____ _____
    b. If yes, how traumatic was this?_____
    c. If yes, how much did you confide in others?___

McGill Pain Questionnaire (MPQ) (Vulvodynia and FMS Groups only)
Some of the words below describe your vulvar/genital pain. Place a check next to **ONLY** those words that best describe it. Leave out any category that is not suitable. Use only a single word in each appropriate category - the one that applies best.

1
Flickering ___
Quivering ___
Pulsing ___
Throbbing ___
Beating ___
Pounding ___

2
Jumping ___
Flashing ___
Shooting ___

3
Pricking ___
Boring ___
Drilling ___
Stabbing ___
Lancinating ___

4
Sharp ___
Cutting ___
Lacerating ___

5
Pinching ___
Pressing ___
Gnawing ___
Cramping ___
Crushing ___

6
Tugging ___
Pulling ___
Wrenching ___

7
Hot ___
Burning ___
Scalding ___
Searing ___

8
Tingling ___
Itchy ___
Smarting ___
Stinging ___

9
Dull ___
Sore ___
Hurting ___
Aching ___
Heavy ___

10
Tender ___
Taut ___
Rasping ___
Splitting ___

11
Tiring ___
Exhausting ___

12
Sickening ___
Suffocating ___

13
Fearful ___
Frightful ___
Terrifying ___

14
Punishing ___
Grueling ___
Cruel ___
Vicious ___
Killing ___

15
Wretched ___
Blinding ___

16
Annoying ___
Troublesome ___
Miserable ___
Intense ___
Unbearable ___

17
Spreading ___
Radiating ___
Penetrating ___
Piercing ___

18
Tight ___
Numb ___
Drawing ___
Squeezing ___
Tearing ___

19
Cool ___
Cold ___
Freezing ___

20
Nagging ___
Nauseating ___
Agonizing ___
Dreadful ___
Torturing ___

PPI
People agree that the following 5 words represent pain of increasing intensity. Which word best describes your vulvar/genital pain? Please check one.

1 Mild ___
2 Discomforting ___
3 Distressing ___
4 Horrible ___
5 Excruciating ___

Pain Catastrophizing Scale (PCS) (Vulvodynia and FMS Groups only)

Directions: Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures, or surgery. If you choose to decline to respond to any item that requires a written response, please write DR in the box.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Please indicate the degree to which you have these thoughts and feelings when you are experiencing the pain you listed above. To indicate your answer, please choose one statement per question.

When I am in pain…

1. I worry all the time about whether the pain will end.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

2. I feel I can't go on.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

3. It's terrible and I think it's never going to get any better.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response

☐ To a great degree
☐ All the time

4. It's awful and I feel that it overwhelms me.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

5. I feel I can't stand it anymore.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

6. I become afraid that the pain will get worse.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

7. I keep thinking of other painful events.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

8. I anxiously want the pain to go away.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

9. I can't seem to keep it out of my mind.

☐ Not at all

☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

10. I keep thinking about how much it hurts.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

11. I keep thinking about how badly I want the pain to stop.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

12. There's nothing I can do to reduce the intensity of the pain.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

13. I wonder whether something serious may happen.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

fMRI Paradigm Anxiety Scale (All groups)
To be verbally reported by the participant before the sham MRI session, before the start of the imaging session, once the imaging session is halfway to completion, and once the imaging session has been completed.

0 = I feel calm and at ease.

10 = I feel as if I may have an anxiety attack.

Rate your anxiety at this moment…

0 1 2 3 4 5 6 7 8 9 10

Pain Catastrophizing Scale (Modified PCS) All groups)

Directions: Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures, or surgery. If you choose to decline to respond to any item that requires a written response, please write DR in the box.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Please indicate the degree to which you have these thoughts and feelings *right* now, that is, *at this moment*. To indicate your answer, please choose one statement per question.

At this moment…

1. I am worried about whether the pain will end.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

2. I feel I can't go on.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

3. It's terrible and I think it's never going to get any better.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

4. It’s awful and I feel that it overwhelms me.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

5. I feel I can’t stand it anymore.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

6. I am afraid that the pain will get worse.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

7. I keep thinking of other painful events.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

8. I anxiously want the pain to go away.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

9. I can’t seem to keep it out of my mind.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response

☐ To a great degree
☐ All the time

10. I keep thinking about how much it hurts.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

11. I keep thinking about how badly I want the pain to stop.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

12. There's nothing I can do to reduce the intensity of the pain.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

13. I am wondering whether something serious may happen.

☐ Not at all
☐ To a slight degree
☐ To a moderate degree ☐ Decline Response
☐ To a great degree
☐ All the time

Appendix H: Debriefing Form

Debriefing Form

Investigation of descending pain modulation in the brain and spinal cord of women with vulvodynia

Thank you very much for your participation in the brain imaging study examining pain processing, psychosocial function, and DNA in women with provoked vestibulodynia (PVD), generalized vulvodynia (GVD), fibromyalgia, and pain-free participant controls. Your contribution to research is essential to progress in this field. For your time and inconvenience, if you have vulvodynia, you will be receiving $75.00 cash, and if you have fibromyalgia or no pain, you will be receiving $60.00 cash. Resources and information on vulvodynia can be found at our website: www.sexlab.ca under our "Education" section. If you have any questions regarding the study or general information on vulvodynia or fibromyalgia, please contact our lab at any time.

As stated previously, all information that you provided throughout the study is confidential. The research team members working directly on this project are the only individuals who have access to your responses, and they will not link your name with your responses. If you provided your email address/phone number to the research team members, it will only be used to contact you if you stated that you wish to be contacted for further studies or if you requested further information about the study or chronic pain conditions. If you wish to withdraw from the study and/or do not wish to have us access your data, please contact the Sexual Health Research Laboratory at (613) 533-3276 or sex.lab@queensu.ca or The Stroman Laboratory at (613) 533-3245 or stromanp@queensu.ca. You may request to have your data withdrawn from the study at any time up until the study has ended (insert anticipated completion date: December 2018), or until the data have been analyzed and/or presented/published, whatever comes first.

Should you have any further questions, comments or concerns, or wish to obtain more information, please do not hesitate to contact Dr. Caroline Pukall (phone: (613) 533-3200; e-mail: caroline.pukall@queensu.ca), Dr. Patrick Stroman in the Stroman Lab (phone: (613) 533-3245; e-mail: stromanp@queensu.ca), the Sexual Health Research Laboratory (phone: 613 533-3276; e-mail: sex.lab@queensu.ca). If you have any concerns about your rights as a research participant please contact Dr. Albert Clark, Chair of the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at (613) 533-6081. If you would like more information about vulvodynia, fibromyalgia, or sexual health or sexual functioning, please visit the following websites or contact our research team.

- **www.sexlab.ca**
- **http://www.sexualityandu.com/index_e.aspx**
- **http://www.nva.org**
- **http://www.niams.nih.gov/health_info/fibromyalgia**

Some of the questions in this survey were sensitive in nature. If you are feeling distressed, please consult the following sources for help:

- **Telehealth Ontario**: 1-866-797-0000
- **Frontenac Community Mental Health Services:** (24 hour crisis line): (613) 544-4229
- **Lanark Leeds and Grenville Mental Health Crisis Line:** 1-866-281-2911
- **Lennox & Addington Community Crisis Centre:** (613) 354-7388
- **Telephone Aid Line Kingston (TALK):** (613) 544-1771