Published in final edited form as:

Pain. 2019 July ; 160(7): 1529–1540. doi:10.1097/j.pain.0000000000001532.

Altered Gray Matter Volume in Sensorimotor and Thalamic Regions associated with Pain in Localized Provoked Vulvodynia: A Voxel-based Morphometry Study

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INTRODUCTION

Provoked vestibulodynia (PVD) is a chronic pain disorder characterized by local hypersensitivity and severe pain with pressure localized to the vulvar vestibule, and is the leading cause of painful intercourse in reproductive-aged women [5; 44]. The underlying pathophysiology of PVD remains largely unknown [61], but PVD often co-exists with other

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Author Contributions:

All authors have read and approved the paper.

Each author made the following contributions:

1. Funding (AR, JSL, EAM, KT, LVO, PE, KT)
2. Study Conceptualization and Design (AG, AR, JSL, EP, PE, LVO)
3. Data Acquisition (JS, SM, EP, LVO, LA)
4. Data Analysis (AG, RB, KH, JSL)
5. Data Interpretation (AG, AR, JSL, RB)
6. Manuscript preparation and Critical Revisions (AG, AR, RB, KT, EAM, JSL, LVO, PE, EP, LA)

Disclosures: No conflicts of interest exist.

chronic pain conditions [3; 47] and mood disorders [36], implicating common underlying mechanisms. [39; 47; 48]

Neuroimaging studies have demonstrated alterations in sensorimotor integration and pain processing regions associated with evoked pain and symptom reports in vestibulodynia [22-24; 45; 51]. Compared to HCs and irritable bowel syndrome (IBS), PVD have 1) microstructural alterations in cortico (primary sensory and motor cortex) - thalamic and basal ganglia tracts involved in sensorimotor integration and pain processing and 2) altered intrinsic resting state functional connectivity in sensorimotor regions, each correlated with evoked pain and muscle tenderness. [22; 23] These findings are consistent with task-based fMRI studies reporting that PVD compared to HCs have greater responses in sensorimotor regions with pressure evoke pain at the vulvar vestibule or to the thumb. [40; 45] Central alterations have also been observed in the default mode, salience, and emotional regulation networks but have not been consistently reported across all brain modalities [40; 45]. Together, research supports the notion that anatomical and functional alterations of the brain, particularly in sensorimotor cortices and subcortical regions associated with sensorimotor integration and pain processing, are closely associated with symptoms, and may be responsible for the hypersensitivity to pain, or are secondary responses to the chronic pain experienced in PVD.

Chronic pain is associated with both decreases and increases in gray matter, with increases most often observed in sensorimotor and basal ganglia regions. [7; 53] Symptom-associated increases in gray matter density in the basal ganglia and the hippocampus have previously been reported in a small sample of PVD patients compared to HCs. [41] The underlying cellular and molecular mechanisms of gray matter change remain unclear and include changes glial cells, axon sprouting, dendritic branching and synaptogenesis, neurogenesis, and angiogenesis, presumably due to increased nociceptive input and resembling learning-associated plasticity [7; 31; 34; 55; 66]. Hypothesized mechanisms underlying the direction of gray matter changes include pain duration (i.e., shorter compared to longer duration of the disorder reflecting early versus later developmental chronic pain states), pain type (e.g., vulvodynia, headache), etiology (central vs peripheral) and confounding factors (e.g., anxiety). [7; 38; 51; 55; 57; 65; 66]

In this study, voxel-based morphometry analyses was applied to specifically test whether the gray matter volumes in regions associated with sensorimotor-thalamic and basal ganglia circuits are altered in PVD. We also tested the replicability of previous reported gray matter increases in the basal ganglia and hippocampus in PVD compared to HCs. Condition-specific alterations in sensorimotor cortices, basal ganglia and thalamus were examined by comparing PVD to another chronic pain condition, irritable bowel syndrome (IBS) [22; 23]. Finally, we hypothesized that gray matter alterations in PVD would be associated with reports of increased pain and muscle tenderness as well as pain duration.

MATERIALS AND METHODS

Participants

The sample comprised healthy control (N=45), PVD (N=45), and IBS (N=29) women. All imaging data was obtained from the NIH-funded Pain and Interoception Imaging Network (PAIN) [32]. A total of 45 healthy control subjects (HCs), 29 from the University of California, Los Angeles (UCLA) and local Los Angeles community and 16 from the University of Leuven (UL) community, were recruited by advertisement and screened via medical exam for absence of other pain disorders.

Women with PVD were recruited through the UCLA Obstetrics and Gynecology Clinic and through an online survey concerning dyspareunia, announcements during the UL Masters courses in Sexology and Psychology and by electronic announcements addressed to the mailbox of all students of the Faculty of Medicine at UL, N=45 (UCLA, n=29, UL, n=16). The diagnosis of PVD was identified during a clinical examination by an OB/GYN at both the study sites, who are experienced at examining women with vulvar pain. Inclusion criteria for patients with PVD were at least 6 months of vulvar vestibular pain at last 4 out of 10 in severity during intercourse and other activities involving vestibular pressure (e.g. tampon use) and findings on exam consistent with vestibulodynia. Infections such as candida, bacterial vaginosis or herpes simplex and other dermatological conditions were ruled out by history, and visual inspection. Additionally, at UCLA, infections were ruled out by vaginal pH and saline and potassium hydroxide slide prep. Speculum examination of the vagina and bimanual pelvic examination were performed to exclude other pathology.

Patients with irritable bowel syndrome (IBS, N=29) were recruited through the UCLA Oppenheimer Center for Neurobiology of Stress and Resilience. The diagnosis of IBS was confirmed using Rome III [46] criteria during a clinical examination by a gastroenterologist or nurse practitioner experienced in functional GI disorders. IBS is defined as recurrent abdominal pain or discomfort for at least three days/month in the last three months and is associated with two or more of the following: 1) improvement with defecation. 2) onset associated with a change in frequency of stool; and 3) onset associated with a change in form (appearance) of stool. To account for the comorbidity between PVD and IBS, presence of IBS symptoms for the PVD subjects and presence of PVD symptoms for the IBS subjects were recorded during the medical history. However, potential PVD subjects were excluded if IBS was distressing or was their most important pain complaint.

Exclusion criteria for all subjects included pregnancy or lactation, substance abuse, tobacco dependence (smoked half a package of cigarettes or more daily), abdominal surgery, current or past psychiatric illness, extreme strenuous exercise (exercise more than one hour per day), and major medical or neurological conditions. In addition, subjects with current regular use of analgesic drugs (including narcotics, opioids, and α 2- δ ligands) were excluded. Use of medications such as antidepressants (low-dose tricyclic anti-depressants, selective serotonin uptake inhibitors, nonselective serotonin reuptake inhibitors) was only allowed if patients had been on a stable dose for a minimum of 3 months. All subjects were right handed and premenopausal confirmed by self-report and were scanned during the follicular phase of the menstrual cycle.

The study was approved by the University of California, Los Angeles (UCLA) Institutional Review Board and the Medical Ethics Committee and Clinical Trial Center of the University Hospitals Leuven and was conducted in accordance with the institutional guidelines regulating human subject research. All subjects provided written informed consent to participate and were compensated for participating in the study.

Clinical Assessments

Clinical Assessment of PVD.—During clinical examination prior to brain imaging detailed information was obtained regarding vulvar pain for the PVD patients. A brief neurosensory examination was conducted [68] on the PVD patients from both sites. Pain testing of the vulva and vestibule was performed using a cotton swab, which is the main diagnostic tool used in PVD studies [18]. The vaginal muscle examination was performed, and the participant was asked to rate the pain severity each site from 0-10/10 (0; none –10; most severe pain imaginable).

PVD subjects from both UCLA and Leuven were mapped for pain in the vulvar vestibule by touching the vestibule perpendicularly with the cotton end of swab (enough to indent the mucosa to a depth of less than 1/3 of the cotton end) for 1 second at 5, 6, 7 (posterior vestibule), 10, and 2 o'clock (peri-urethral). Participants were asked to rate the pain at each site from 0-10/10. A total score was calculated by adding the ratings across vulvar sites.

At UCLA only, internal muscle tone and tenderness was assessed with a single lubricated digit, applying approximately 2 kg of pressure for 2 seconds. The examiner's finger pressure was calibrated immediately before the exam with an algometer. The bulbocavernosus muscles at 5 and 7 o'clock (muscles at the vaginal entrance) and the levator ani complex (in the vagina) were assessed in the midline and laterally at 5 and 7 o'clock. A total score was calculated by adding the ratings across muscle sites.

PVD patients from UCLA also filled out the Modified Gracely General Vaginal Pain Severity Scale [21; 69]. Average, lowest and highest level of pain related to sex and not related to sex was also recorded (scale 0-100, 0= no pain, 100= most intense pain imaginable). Pain duration was only available for women with PVD at UCLA.

Clinical Assessment of IBS.—Patients with IBS who had all types of predominant bowel habits were included. Overall GI symptom severity, abdominal pain, and bloating pain in the past week were assessed using a 21-point Numerical Rating Scale (scale=0 - 20, 0 = no pain and 20 =the most intense symptoms imaginable). Usual symptom severity was assessed on an ordinal scale where 1 = None, 2 = Mild, 3 = Moderate, 4 = Severe, and 5 = Very Severe. Age of onset was also noted including duration of symptoms in years.

Questionnaires

Levels of trait anxiety were assessed in all 119 subjects using the State-Trait Anxiety Inventory (STAI) a self-report 20-item instrument, which evaluates the general propensity towards anxiety including aspects of calmness, confidence, and security on a frequency scale: 1. Almost never, 2. Sometimes, 3. Often, and 4. Almost always. Scoring ranges from 20 to 80 with higher scores indicating greater levels of trait anxiety. Normative data indicate

the standardized trait score for neuropsychiatric and general medical surgery patients ranging from 40 to 54 [56]. Norms specific to chronic pain have not been established.

MRI: Data Acquisition & Data Preparation

Data Acquisition—For MRI scans acquired at UCLA, whole brain data was acquired using a 3.0T MRI scanner (Siemens Trio; Siemens, Erlangen, Germany) after a sagittal scout was used to position the head. Structural scans were obtained from two different acquisition sequences using a high-resolution 3-dimensional T1-weighted, sagittal magnetization-prepared rapid gradient echo (MP-RAGE) protocol: Protocol 1) repetition time (TR) = 2200ms, echo time (TE) = 3.26ms, structural acquisition time (TA)=10m13s, slice thickness = 1mm, 176 slices, 256*256 voxel matrix, 1mm voxel size. Protocol 2) repetition time (TR) = 2300ms, echo time (TE) = 2.98ms, structural acquisition time (TA)=5m12s, slice thickness = 1mm, 176 slices, 256*256 voxel matrix, 1mm voxel size.

For MRI scans acquired at Leuven, whole brain data was acquired using a 3.0T MRI scanner (Philips Achieva; Philips, Amsterdam, Netherlands) after a sagittal scout was used to position the head. Structural scans were obtained from an acquisition sequence using a high-resolution 3-dimensional, T1-contrast enhanced turbo field echo (3D-T1-TFE) protocol: repetition time (TR) = 9.600ms, echo time (TE) = 4.60ms, structural acquisition time (TA)=6m23s, slice thickness = 1mm, 182 slices, 256*256 voxel matrix, 1mm voxel size.

Data Preparation—Raw structural images for the Leuven data that originated from the Philips MRI scanner was converted from PAR/REC to the NIFTI format using the conversion script created by the Dartmouth imaging group (dbic.dartmouth.edu/wiki/index.php/Imaging_Data_Formats). Careful considerations were made and scans were manually inspected for known misalignment issues that would arise after transforming “world” space from Philips scanners to “voxel” space used by the NIFTI format.

Data Analysis: Image Processing and Data Analysis.

Voxel-Based Morphometry—Voxel-Based Morphometry analyses were conducted in FSL-VBM which is a part of the FSL software package [29]. Brain extraction and segmentation from raw MP-RAGE scans was done using the *fsl_anat* script in FSL. The following steps were done using this script. All images were first reoriented to standard MNI orientation. Brain extraction was then conducted on all subject's T1 weighted MP-RAGE images using the robust version BET command, which calls the command multiple times to move the center-of-gravity closer to the true center and extracts out only the brain of the image. Afterwards, all images were checked manually to determine if the brain was adequately extracted and non-brain matter was removed. If the extraction was inadequate, which included making sure all the dura mater and skull were removed, the BET process was manually altered for each brain by changing fractional intensity threshold and/or its vertical gradient until only the whole brain was included. Following this process, all extracted brain images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF).

A study specific GM template was created using non-linear registration onto the Montreal Neurological Institute standard space (MNI-152) using FNIRT [2]. To limit bias in the template and ensure equal group representation, only a random subset of PVD (n=29) and HC (N=29) images (N=29) and all IBS images were used to create the template[10].

All of the GM images were non-linearly registered to the study specific template, modulated by dividing them by the Jacobian of the warp field – to correct for local expansion or contraction – [20] and concatenated to create a 4D image. The modulated images were then smoothed with an isotropic Gaussian kernel with a standard deviation of 3 mm. A mask was created to investigate voxel-wise differences between PVD patients and controls in specific regions of interest (see Table 1, Figure 1). In order to restrict statistical test to our hypotheses that women with PVD (compared to HCs and IBS) would show alterations in sensorimotor cortices and subcortical regions associated with sensorimotor integration and pain processing, we created a mask comprised of sensorimotor [primary somatosensory cortex/S1 (postcentral gyrus and sulcus, central sulcus, primary motor cortex/M1 (precentral gyrus, inferior and superior parts of the precentral sulcus), secondary somatosensory cortex/S2 (subcentral gyrus/central operculum and sulci), and supplementary motor area, posterior insula (primary viscerosensory cortex)] and subcortical [basal ganglia (caudate nucleus, pallidum, nucleus accumbens, putamen), hippocampus, and thalamus] regions. The mask containing these regions was created using ROIs from the Destrieux atlas [8], the Harvard-Oxford Subcortical Atlas [17], and an in-house parcellation of the posterior insula and the supplementary motor area. The anterior and posterior long gyri comprised this ROI. The circular sulcus served as the posterior margin [37]. The SMA was defined as the area in the superior frontal gyrus which was dorsal to the cingulate sulcus, anterior to the primary motor cortex, through the vertical line transversing the anterior commissure, and posterior to the virtual line passing through the genu of the corpus callosum.[30; 42; 43]. The final inclusionary mask was created by adding the ROIs together using *fslmaths*. Following the construction of the mask, anterior portions of the middle frontal gyrus and inferior frontal sulcus were manually erased to only include portions of the supplementary motor cortex. Compared to a whole brain gray matter segment(2,081,768 mm³ and 260,221 voxels, this mask reduced limited the search space to 191,200 cubic mm³ and comprised 23,900 voxels, effectively increasing power for detecting differences in our apriori hypothesized differences. Finally, to test for group differences a voxel-based general linear model (GLM) controlling for age and site (Leuven and UCLA) was applied, with permutation based testing at 5000 iterations used to create clusters with the Threshold Free Cluster Enhancement (TFCE) method using *randomise* in FSL [54]. Group differences between PVD and IBS were only computed using the sample of PVD women from UCLA. We did not include the Leuven sample in this contrast as IBS women were not included in the Leuven study and we would not be able to control for the influence of site. TCFE relieves the need for setting an arbitrary threshold, which can be problematic as small variations in the data around a hard threshold can bias and have a large impact on the final results. To summarize the TFCE methodology, each voxel's statistic value is transformed based on the height and spatial contiguity of neighboring voxels to create the TFCE statistic value. Significance was considered at $p < .05$, corrected for multiple comparisons using the False-Discovery Rate (FDR) method. All FDR correction was done using the *fdr* program within FSL using the

uncorrected p-maps output from *randomise* based on the TFCE statistic. Images of the results were produced using Mango and BrainNet [62] software.

Data analysis of non-imaging data.—Means and standard deviations of clinical and behavioral measures were calculated for each disease group. Means and standard deviations were also calculated for study variables specific to the symptoms of PVD and IBS subjects. Partial correlations controlling for site and age with the full sample of PVD were run to determine the relationship between vulvar pain, trait anxiety, and GMV of regions that were altered in PVD. Partial correlations controlling for age were run with just the UCLA PVD sample to determine the relationship between vulvar pain, muscle tenderness and pain with intercourse, as these measures were not collected at both Leuven and UCLA. The partial correlations were run using the cluster maxima for each cluster from the smoothed modulated images. These were extracted for each subject using *fslmeants*. All partial correlations were run in SPSS 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.).

Estimated power for correlational analyses based on sample size.—Sensitivity analyses in G*Power[14] indicated that the sample size of 58 PVD from both research sites provides adequate (80%) power to detect a correlation, $r \geq .35$ using at alpha levels of .05 (p uncorrected) or $r \geq .45$, using Bonferroni adjustment for multiple comparisons (12 tests, $\alpha = .004$), if these associations exist. In the smaller sample of 29 PVD at UCLA, has adequate power to detect a correlation as large as $r \geq .47$ at uncorrected alpha levels of .05 or $r \geq .60$ using Bonferroni adjustment for multiple comparisons ($\alpha = .004$), if these associations exist. We reported the correlations estimates with 99% confidence intervals, and p values for any correlation reaching significance at $p < .10$. We recommend placing interpretive emphasis be on the estimated association effect sizes and confidence interval, rather than significance testing.

RESULTS

Clinical and behavioral characteristics

PVD (N=45, mean age = 27.67 y, SD = 6.65, range = 19-52 y) did not differ in age compared to IBS (N=29, mean age = 29.00 y SD = 8.98, range = 23-43 y), and HC (N=45, mean age = 25.27 y, SD = 5.83, range = 18-44 y); PVD vs HC: $t(88) = 1.82$, $p = 0.07$; PVD vs IBS: $t(72) = 0.73$, $p = 0.47$).

Levels of trait anxiety in PVD were higher compared to HCs ($p = .0003$) and lower compared to IBS ($p = .023$), HC (mean (SD) = 42.4(7.1); PVD = 49.7(10.3), and IBS=54.8(10.4).

Values for vulvar vestibular pain as assessed by cotton swab (collected at both UCLA and Leuven) and for vaginal muscle tenderness (collected at UCLA) as assessed by finger pressure exam for the PVD subjects are summarized in Table 2A. The average duration of PVD pain of patients at UCLA was about 88.7 months (7.4 y). For the PVD subjects the average level of pain intensity of patients at UCLA during the past 24 hours was 5.90 (SD=4.84), and average level of pain unpleasantness of patients at UCLA during the past 24 hours was 5.29 (SD=4.35). The values for abdominal pain and gastrointestinal symptoms for

the IBS subjects are summarized in Table 2B. The average symptom duration for IBS subjects was 8.7 years. None of the 29 IBS patients reported comorbid PVD symptoms.

Disease related structural brain differences between PVD patients and HC subjects

Structural brain differences between PVD patients and HC subjects are summarized in Table 3 and depicted in Figure 2. Compared to HCs, PVD had significantly greater GMV bilaterally in the hippocampus ($q_{\text{left}}=.01$, $q_{\text{right}}=.001$) and the basal ganglia ($q_{\text{left}}=.002$, $q_{\text{right}}=.004$). The basal ganglia clusters were comprised by caudate (the maxima for both clusters) and extending into the nucleus accumbens and the putamen (on the right).

In line with our hypotheses, PVD compared to HCs showed significantly greater GMV bilaterally in sensory and motor cortices. PVD patients exhibited greater volume of the bilateral postcentral gyrus ($q_{\text{left}}=.01$, $q_{\text{right}}=.01$) along the dorsal portion innervating the genital area/pelvic floor, and bilateral precentral gyrus ($q_{\text{left}}=.02$, $q_{\text{right}}=.02$). No brain regions were smaller in PVD compared to HCs.

Disease related structural brain differences between PVD patients and IBS patients

Structural differences between PVD patients and IBS patients are summarized in Table 4 and depicted in Figure 3, Figure 4. Greater GMV was observed in PVD compared to IBS in the sensorimotor network (left supplementary motor area ($q=.001$), bilateral posterior insula ($q_{\text{left}}=.009$, $q_{\text{right}}=2 \times 10^{-4}$), left precentral gyrus ($q=2 \times 10^{-4}$) along the ventral portion innervating facial regions, right postcentral gyrus ($q=.004$) along the dorsal portion innervating the genital/pelvic floor area, as well bilateral hippocampus ($q_{\text{left}}=8 \times 10^{-4}$, Right: $q_{\text{right}}=2 \times 10^{-4}$), network. Compared to IBS, PVD had lower GMV observed bilaterally in the thalamus (Left: $q_{\text{left}}=2 \times 10^{-4}$, Right: $q_{\text{right}}=2 \times 10^{-4}$), and left precentral gyrus ($q=.002$) = at the ventral portion innervating facial regions.

Gray Matter Correlations with Clinical Symptoms

Figure 5 depicts the scatterplots for the partial correlations. No observed correlation maintained significance after applying a stringent Bonferroni threshold for multiple comparisons.

UCLA and Leuven PVD Samples.—In PVD, volume in the left posterior insula (PVD > IBS; $r_{(38)} = -.40$ (99% CI = -0.01 , -0.69), $p_{\text{uncorrected}(unc)} = .01$) was negatively associated with total vulvar pain scores after controlling for site and age. No correlations with the STAI were observed.

UCLA PVD sample.—Pain with intercourse was positively associated with GMV in the right precentral gyrus (PVD > HC, average level $r_{(24)} = .36$ (-0.15 , 0.72), $p_{\text{unc}} = .07$), highest level, $r_{(24)} = .35$ (-0.16 , 0.71), $p_{\text{unc}} = .08$, and lowest level $r_{(24)} = .36$ (-0.15 , 0.72), $p_{\text{unc}} = .06$). GMV in the right hippocampus was also positively associated with the lowest level of pain with intercourse (PVD > IBS; $r_{(24)} = .46$ (-0.03 , 0.77), $p_{\text{unc}} = .02$). On the other hand negative associations were observed between GMV in the left caudate and pain with intercourse (PVD > HC; average level $r_{(24)} = -.40$ (-0.74 , 0.10), $p_{\text{unc}} = .04$, highest level, $r_{(24)} = -.44$ (-0.76 , 0.06), $p_{\text{unc}} = .02$). Total muscle tenderness scores positively associated with

GMV in the left postcentral gyrus (PVD >HC; $r_{(25)} = .35(-0.15, 0.71)$, $p_{unc} = .07$), but tended to be negatively associated with GMV in the right caudate (PVD >HC, $r_{(25)} = -.36(-0.71, 0.14)$, $p_{unc} = .06$).

DISCUSSION

This voxel-based morphometry study demonstrated alterations in gray matter volumes in brain regions associated with sensorimotor, cortico-thalamic, and basal ganglia circuits in PVD, compared to both healthy and disease control groups. PVD patients compared to HCs showed greater GMVs in the basal ganglia, somatosensory and motor cortices and the hippocampus. When comparing PVD and IBS, distinct alterations were observed in sensorimotor cortices, posterior insula and thalamus. Finally, regional GMV alterations, showed associations with key clinical outcomes. These findings confirm previously reported increases in the hippocampus and basal ganglia regions in PVD compared to HCs.

Hippocampus volume increased in PVD

Compared to HCs and IBS, women with PVD had higher bilateral hippocampal GMVs that were associated with increased ratings of pain during intercourse. Alterations in the fiber tracts extending from the hippocampus to hypothalamus and the thalamus have previously been reported in PVD[23]. Interestingly, hippocampal volume has been reported as negatively associated with basal cortical levels in chronic pain[59]. Thus, hippocampal alterations may be associated with the blunted morning awakening cortisol reported in vulvodynia [11; 25]. Also, elevation of inflammatory cytokines in PVD including IL-6, and tumor necrosis factor alpha [TNF- α] have been inconsistently reported in small samples[1; 4; 12; 13; 15; 16; 51; 52; 60]. Thus, dysregulation of the HPA axis [35] and/or stress induced microglia responses, which produce TNF- α , and IL-6[41; 51], may underlie the observed alterations in hippocampal volumes and merit further investigation.

Basal ganglia volumes increased in PVD

Women with PVD compared to HCs had greater GMV bilaterally in the basal ganglia clusters comprised of the caudate nucleus, putamen, nucleus accumbens. Greater caudate volumes were associated with lower ratings of pain during intercourse, lower minimum levels of pain during intercourse, and lower levels of muscle tenderness. Greater GMV in the basal ganglia has been reported for a variety of chronic pain conditions including back pain [49], fibromyalgia [50] and vestibulodynia [51]. Increases in caudate nucleus activity have been observed during the anticipation and experience of vestibular pain. The basal ganglia are a site of multisensory integration and an integrative area for pain processing [6; 40]. In addition, the majority of neurons in the striatum (includes caudate, nucleus accumbens, putamen) are medium spiny neurons (MSNs) [63], with GABAergic inhibitory projections that eventually reach the thalamus via signaling of the globus pallidus and the substantia nigra [63]. Greater GMV in the basal ganglia may reflect neuroplastic increases in GABAergic projections needed to inhibit vulvar pain. Greater resting-state functional connectivity has also been observed in the basal ganglia compared to HCs, and this greater connectivity is associated with lower vulvar pain [22], providing further support that greater activity in a region with greater amounts of MSNs may be associated with lower clinical

pain. This explanation is consistent with the association between increased caudate volume and lower reported levels of pain during intercourse. Future studies using molecular neuroimaging are needed to confirm this hypothesis.

As hypothesized and in line with previous research [22; 24; 45], differences in GMV were observed in sensorimotor regions between patients with PVD and HCs, as well as PVD and patients with IBS. Specifically, PVD patients compared to both HC and IBS had greater GMV in the precentral gyrus and dorsal postcentral gyrus, and PVD patients had greater GMV in the supplementary motor area (SMA) compared to IBS. The cluster showing differences in PVD compared to the other two groups was localized to an area of the medial primary somatosensory cortex associated with innervating the thumb/fingers/hand [9], which is consistent with reports of lower pain thresholds to a painful thumb stimulus in vestibulodynia [24]. This hypothesized generalized sensitivity to non-vulvar pain may be due, in part, to central alterations in somatosensory cortex.

In PVD compared to IBS, GMV in the precentral gyrus was greater in the part served by the corticobulbar tract *and* corticospinal tract (eventually merging into the pyramidal tracts), but lower in the part served by the corticospinal tract. These pyramidal tracts send signals which terminate in the brainstem (corticobulbar) or the spinal cord (corticospinal). Different signaling mechanisms between sensorimotor cortices involving the influence of varying tracts may differentiate PVD from IBS and may underlie differences in GMV observed here [19]. Our correlational results revealed trends for positive associations between GMV in the somatosensory and motor cortices and various measures of vulvar pain and muscle tenderness. Future research using high resolution imaging of the brainstem and spinal cord is needed to understand the underlying nociceptive pathways contributing to different chronic pain conditions.

The thalamus only showed differences in GMV when comparing IBS and PVD, with IBS exhibiting greater GMV. Thalamic nuclei have direct reciprocal connections with sensory, limbic and motor cortices, and remain responsive to peripheral and visceral noxious stimuli even under anesthetized conditions, indicating their crucial role in the transmission of nociceptive stimuli [64]. As differences in the thalamus was observed when comparing the two chronic pain conditions, it is conceivable that the different temporal patterns of afferent input to the brain in the two chronic pain conditions result in different neuroplastic changes.

The volumetric alterations in the basal ganglia and somatosensory regions parallel findings reported in other brain imaging modalities. Women with PVD show microstructural alterations consistent with an increased strength of axonal projections and increased myelination in sensorimotor, corticothalamic, and basal ganglia circuits involved in sensorimotor integration and pain processing [23]. Also, alterations in resting-state functional connectivity between sensorimotor and basal ganglia regions have been observed in PVD and are highly correlated with pain sensitivity and muscle tenderness [22]. In addition, in response to vestibular stimulation, PVD subjects show greater activation in sensorimotor regions including supplemental motor area, secondary somatosensory cortex, and thalamus. These structural and functional alterations could result from hyper-innervation

of the vulvar vestibule [58] or increased noxious input, or from greater strength of axonal or dendritic projections to specific areas of primary sensorimotor cortices [22; 23].

Ultimately the observed differences between IBS and PVD in sensorimotor and thalamic regions may be associated with the nature of the chronic pain, visceral compared to somatic. Recent work suggests that somatotopic reorganization in chronic pain is present [1; 26; 27; 56], which can lead to altered responses to sensory stimuli. Our findings provide specific sensorimotor seed region coordinates for mapping the topographical organization of connectivity between the sensorimotor regions and regions comprising emotional, salience, and default mode networks in PVD. Future research is needed to determine if differences the topographical organization of connectivity [28] within the sensorimotor system are present in different chronic pain conditions.

Replication and extension studies

Given that neuroimaging studies of chronic pain often have small sample sizes which are known to result in inflated effect sizes, the importance of replication studies cannot be overemphasized[33]. This study represents a conceptual replication of a study examining gray matter differences in 14 women with vulvodynia and 14 HCs using a 1.5 Tesla scanner[51]. In the current study, structural images were acquired in a 3 Tesla scanner providing greater spatial resolution. Leveraging data from the PAIN repository[25], the sample size was tripled providing greater statistical power. Overall, our results partially confirmed the previously reported volumetric increases in the basal ganglia and the hippocampus in PVD compared to HCs.

Limitations and Directions for Future Research

There are several limitations to the study: Individuals with comorbid chronic overlapping pain conditions, and psychiatric disorders were excluded from the study, thus inferences may not generalize to more severe clinical presentations. Also, pain with intercourse was assessed using self-report. Alternatively, a standardized tampon insertion and removal test may allow greater construct validity. Given the cross-sectional design of the study, it is not possible to determine whether differences in GMV are a result of, or caused, the painful symptoms. Ultimately, T1-weighted structural images only provide nonspecific assessment of underlying tissue characteristics[67] and further research is needed to determine the exact histological underpinning of gray matter changes observed in this study. Finally, although evidence was found for both common and distinct gray matter alterations in PVD compared to IBS, it is important to extend this research to include other visceral and somatic chronic pain conditions as well as individuals meeting diagnostic criteria for two or more chronic pain conditions[1].

Summary and conclusions

We found increases in GMV in PVD compared to HCs in sensorimotor, corticothalamic, and basal ganglia regions involved in sensorimotor integration and pain processing. These increases could be likely due to increased strength of axonal or dendritic projections and increased myelination, or alternatively they could also be due to increase in glial cell activation. We also found evidence supporting CMV difference in patients with a chronic

somatic and visceral pain condition. Even though the current findings do not address the question of causality, the correlation of pain sensitivity with regional alterations provides evidence for a possible role of central mechanisms in the generation of PVD symptoms. Longitudinal studies examining the natural course of this disease as well as neural correlates of successful treatment response are critical to answer these questions.

Acknowledgments

Funding Sources: This research was supported in part by grants from the National Vestibulodynia Association (AR/EM) and National Institutes of Health: R01 HD076756 (AR/JSL), P30 DK041301 (EM), R01 DK048351 (EM), P50DK64539 (EM), R01 AT007137 (KT). Pilot scans were provided by the Ahmanson-Lovelace Brain Mapping Center, UCLA

References

- [1]. Akopians AL, Rapkin AJ. Vulvodynia: The Role of Inflammation in the Etiology of Localized Provoked Pain of the Vulvar Vestibule (Vestibulodynia). *Semin Reprod Med* 2015;33(4):239–245. [PubMed: 26132928]
- [2]. Andersson JLR, Jenkinson M, Smith S. Non-linear registration, aka spatial normalisation, 2010. pp. FMRIB technical report TR07JA02-FMRIB technical report TR07JA02.
- [3]. Arnold LD, Bachmann GA, Rosen R, Kelly S, Rhoads GG. Vulvodynia: Characteristics and associations with comorbidities and quality of life. *Obstetrics and Gynecology* 2006;107(3):617–624. [PubMed: 16507933]
- [4]. Baker DA, Peresleni T, Kocis C. Inflammatory Markers in Vestibulodynia. *Obstetrics and Gynecology* 2016;127:1s–2s.
- [5]. Bornstein J, Goldstein AT, Stockdale CK, Bergeron S, Pukall C, Zolnoun D, Coady D, Bornstein J, Goldstein A, Zolnoun D, Bachmann GA, Bissonnette I, Bergeron S, Starke NB, Burrows L, Coady D, Dellon AL, Farmer M, Foster D, Fox S, Goldstein I, Gracely R, Haefner HK, Kellogg-Spadt S, Marvel R, Barracco MM, Morrison P, Parish S, Prendergast S, Pukall C, Reed B, Stockdale C, Boardman L, Goldstein L, Mate P. 2015 ISSVD, ISSWSH, and IPPS Consensus Terminology and Classification of Persistent Vulvar Pain and Vulvodynia. *Journal of Sexual Medicine* 2016;13(4):607–612. [PubMed: 27045260]
- [6]. Borsook D, Upadhyay J, Chudler EH, Becerra L. A key role of the basal ganglia in pain and analgesia - insights gained through human functional imaging. *Molecular Pain* 2010;6(1):27–27. [PubMed: 20465845]
- [7]. Cauda F, Palermo S, Costa T, Torta R, Duca S, Vercelli U, Geminiani G, Torta DM. Gray matter alterations in chronic pain: A network-oriented meta-analytic approach. *NeuroImage Clinical* 2014;4:676–686. [PubMed: 24936419]
- [8]. Destrieux C, Fischl B, Dale A, Halgren E. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. *NeuroImage* 2010;53:1–15. [PubMed: 20547229]
- [9]. Di Noto PM, Newman L, Wall S, Einstein G. The Hermunculus: What Is Known about the Representation of the Female Body in the Brain? *Cereb Cortex* 2013;23(5):1005–1013. [PubMed: 22510528]
- [10]. Douaud G, Smith S, Jenkinson M, Behrens T, Johansen-Berg H, Vickers J, James S, Voets N, Watkins K, Matthews PM, James A. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain* 2007;130:2375–2386. [PubMed: 17698497]
- [11]. Ehrstrom S, Kornfeld D, Rylander E, Bohm-Starke N. Chronic stress in women with localised provoked vulvodynia. *J Psychosom Obstet Gynaecol* 2009;30(1):73–79. [PubMed: 19308786]
- [12]. Eva LJ, Rolfe KJ, MacLean AB, Reid WMN, Fong ACWT, Crow J, Perrett CW. Is localized, provoked vulvodynia an inflammatory condition? *Journal of Reproductive Medicine* 2007;52(5):379–384. [PubMed: 17583235]
- [13]. Falsetta ML, Foster DC, Woeller CF, Pollock SJ, Bonham AD, Haidaris CG, Stodgell CJ, Phipps RP. Identification of novel mechanisms involved in generating localized vulvodynia pain. *Am J Obstet Gynecol* 2015;213(1):38 e31–12. [PubMed: 25683963]

- [14]. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* 2009;41(4):1149–1160. [PubMed: 19897823]
- [15]. Foster DC, Falsetta ML, Woeller CF, Pollock SJ, Song K, Bonham A, Haidaris CG, Stodgell CJ, Messing SP, Iadarola M, Phipps RP. Site-specific mesenchymal control of inflammatory pain to yeast challenge in vulvodynia-afflicted and pain-free women. *Pain* 2015;156(3):386–396. [PubMed: 25679469]
- [16]. Foster DC, Hasday JD. Elevated tissue levels of interleukin-1 beta and tumor necrosis factor-alpha in vulvar vestibulitis. *Obstet Gynecol* 1997;89(2):291–296. [PubMed: 9015038]
- [17]. Frazier JA, Chiu S, Breeze JL, Nikos Makris MPH, Lange N, David Kennedy SN, Herbert MR, Bent EK, Vamsi Koneru BK, Megan Dieterich BE, Steven Hodge BM, Scott Rauch ML, Ellen Grant P, Cohen BM, Seidman LJ, Caviness VS, Biederman J. Structural Brain Magnetic Resonance Imaging of Limbic and Thalamic Volumes in Pediatric Bipolar Disorder. *Am J Psychiatry* 2005;162(7):1256–1265. [PubMed: 15994707]
- [18]. Friedrich EG. Vulvar vestibulitis syndrome. *J Reprod Med* 1987;32(2):110–114. [PubMed: 3560069]
- [19]. Furness JB. The enteric nervous system and neurogastroenterology. *Nature Reviews Gastroenterology & Hepatology* 2012;9:286–294. [PubMed: 22392290]
- [20]. Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ. A Voxel-Based Morphometric Study of Ageing in 465 Normal Adult Human Brains. *NeuroImage* 2001;14(1): 21–36. [PubMed: 11525331]
- [21]. Gracely RH. Evaluation of multi-dimensional pain scales. *Pain*, Vol. 48, 1992 pp. 297–300. [PubMed: 1594252]
- [22]. Gupta A, Rapkin AJ, Gill Z, Kilpatrick L, Fling C, Stains J, Masghati S, Tillisch K, Mayer EA, Labus JS. Disease-Related Differences in Resting State Networks: A Comparison between Localized Provoked Vulvodynia, Irritable Bowel Syndrome, and Healthy Control Subjects. *Pain* 2015;156(5):809–819. [PubMed: 25735001]
- [23]. Gupta A, Woodworth DC, Ellingson BM, Rapkin AJ, Naliboff B, Kilpatrick LA, Stains J, Masghati S, Tillisch K, Mayer EA, Labus JS. Disease-Related Microstructural Differences in the Brain in Women With Provoked Vestibulodynia. *The Journal of Pain* 2018;19(5):528.e521–528.e515. [PubMed: 29391213]
- [24]. Hampson JP, Reed BD, Clauw DJ, Bhavsar R, Gracely RH, Haefner HK, Harris RE. Augmented Central Pain Processing in Vulvodynia. *The Journal of Pain* 2013;14(6):579–589. [PubMed: 23578957]
- [25]. Heim C, Ehler U, Hanker JP, Hellhammer DH. Psychological and endocrine correlates of chronic pelvic pain associated with adhesions. *J Psychosom Obstet Gynaecol* 1999;20(1):11–20. [PubMed: 10212883]
- [26]. Hotz-Boendermaker S, Marcar VL, Meier ML, Boendermaker B, Humphreys BK. Reorganization in secondary somatosensory cortex in chronic low back pain patients. *Spine* 2016;41(11):E667–E673. [PubMed: 27244113]
- [27]. Irimia A, Labus JS, Torgerson CM, Horn JDV, Mayer EA. Altered viscerotopic cortical innervation in patients with irritable bowel syndrome. *Neurogastro Motil* 2015;27(8):1075–1081.
- [28]. Jbabdi S, Sotiropoulos SN, Behrens TE. The topographic connectome. *Curr Opin Neurobiol* 2013;23(2):207–215. [PubMed: 23298689]
- [29]. Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. FSL. *NeuroImage* 2012;62:782–790. [PubMed: 21979382]
- [30]. Kim JH, Lee JM, Jo HJ, Kim SH, Lee JH, Kim ST, Seo SW, Cox RW, Na DL, Kim SI, Saad ZS. Defining functional SMA and pre-SMA subregions in human MFC using resting state fMRI: Functional connectivity-based parcellation method. *NeuroImage* 2010;49(3):2375–2386. [PubMed: 19837176]
- [31]. Labus J, Dinov I, Jiang Z, Ashe-McNalley C, Zamanyan A, Shi Y, Hong J-Y, Gupta A, Tillisch K, Gutman B, Ebrat B, Hobel S, Joshi S, Thompson P, Toga A, Mayer E. Irritable Bowel Syndrome in female patients is associated with alterations in structural brain networks. *Pain* 2014;155(1):137–149. [PubMed: 24076048]

- [32]. Labus JS, Naliboff B, Kilpatrick L, Liu C, Ashe-Mcnalley C, Dos Santos IR, Alaverdyan M, Woodworth D, Gupta A, Ellingson BM, Tillisch K, Mayer EA. Pain and Interoception Imaging Network (PAIN): A multimodal, multisite, brain-imaging repository for chronic somatic and visceral pain disorders. *NeuroImage* 2016;124(Pt B):1232–1237. [PubMed: 25902408]
- [33]. Lalor EC, Raizada RDS, Peelle JE, Evans S. What Has Replication Ever Done for Us? Insights from Neuroimaging of Speech Perception. *Frontiers in Human Neuroscience* 2017;11(41):eCollection 2017–eCollection 2017.
- [34]. Lerch JP, Yiu AP, Martinez-Canabal A, Pekar T, Bohbot VD, Frankland PW, Henkelman RM, Josselyn SA, Sled JG. Maze training in mice induces MRI-detectable brain shape changes specific to the type of learning. *NeuroImage* 2011;54(3):2086–2095. [PubMed: 20932918]
- [35]. McEwen BS, Kalia M. The role of corticosteroids and stress in chronic pain conditions. *Metabolism* 2010;59 Suppl 1:S9–15. [PubMed: 20837196]
- [36]. Meana M, Binik YM, Khalife S, Cohen D. Psychosocial correlates of pain attributions in women with dyspareunia. *Psychosomatics* 1999;40(6):497–502. [PubMed: 10581978]
- [37]. Naidich TP, Kang E, Fatterpekar GM, Delman BN, Gultekin SH, Wolfe D, Ortiz O, Yousry I, Weismann M, Yousry TA. The Insula: Anatomic Study and MR Imaging Display at 1.5 T. *American Journal of Neuroradiology* 2004;25(2):222–232. [PubMed: 14970021]
- [38]. Neeb L, Bastian K, Villringer K, Israel H, Reuter U, Fiebach JB. Structural Gray Matter Alterations in Chronic Migraine: Implications for a Progressive Disease? *Headache* 2017;57(3):400–416. [PubMed: 28028808]
- [39]. Nguyen R, Veasley, Smolenski. Latent class analysis of comorbidity patterns among women with generalized and localized vulvodynia: preliminary findings. *Journal of Pain Research* 2013:303–303. [PubMed: 23637555]
- [40]. Pazmany E, Ly HG, Aerts L, Kano M, Bergeron S, Verhaeghe J, Peeters R, Tack J, Dupont P, Enzlin P, Oudenhove LV. Brain responses to vestibular pain and its anticipation in women with Genito-Pelvic Pain/Penetration Disorder. *NeuroImage: Clinical* 2017;16:477–490. [PubMed: 28932680]
- [41]. Pekny M, Wilhelmsson U, Pekna M. The dual role of astrocyte activation and reactive gliosis. *Neurosci Lett* 2014;565:30–38. [PubMed: 24406153]
- [42]. Picard N, Strick PL. Motor areas of the medial wall: A review of their location and functional activation. *Cereb Cortex* 1996;6(3):342–353. [PubMed: 8670662]
- [43]. Picard N, Strick PL. Imaging the premotor areas. *Curr Opin Neurobiol* 2001;11(6):663–672. [PubMed: 11741015]
- [44]. Pukall CF, Goldstein AT, Bergeron S, Foster D, Stein A, Kellogg-Spadt S, Bachmann G. Vulvodynia: Definition, Prevalence, Impact, and Pathophysiological Factors. *The Journal of Sexual Medicine* 2016;13(3):291–304. [PubMed: 26944461]
- [45]. Pukall CF, Strigo IA, Binik YM, Amsel R, Khalifé S, Bushnell MC. Neural correlates of painful genital touch in women with vulvar vestibulitis syndrome. *PAIN* 2005;115(1-2):118–127. [PubMed: 15836975]
- [46]. Quigley EMM, Drossman DA, Kellow JE. Introduction. The Rome Foundation and Rome III, Vol. 19, 2007 pp. 783–797.
- [47]. Reed BD, Harlow SD, Sen A, Legocki LJ, Edwards RM, Arato N, Haefner HK. Prevalence and demographic characteristics of vulvodynia in a population-based sample, Vol. 206, 2012.
- [48]. Reed BD, Legocki LJ, Plegue MA, Sen A, Haefner HK, Harlow SD. Factors associated with vulvodynia incidence. *Obstetrics and Gynecology* 2014;123(2):225–231. [PubMed: 24402591]
- [49]. Schmidt-Wilcke T, Leinisch E, Gänßbauer S, Draganski B, Bogdahn U, Altmeyen J, May A. Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. *Pain* 2006;125(1-2):89–97. [PubMed: 16750298]
- [50]. Schmidt-Wilcke T, Luerding R, Weigand T, Jü Rgens T, Schuierer G, Leinisch E, Bogdahn U. Striatal grey matter increase in patients suffering from fibromyalgia – A voxel-based morphometry study. *PAIN* 2007;132:S109–S116. [PubMed: 17587497]
- [51]. Schweinhardt P, Kuchinad A, Pukall CF, Bushnell MC. Increased gray matter density in young women with chronic vulvar pain. *Pain* 2008;140(3):411–419. [PubMed: 18930351]

- [52]. Seckin-Alac E, Akhant SE, Bastu E, Tuzlalik S, Yavuz E. Elevated tissue levels of tumor necrosis factor-alpha in vulvar vestibulitis syndrome. *Clin Exp Obstet Gynecol* 2014;41(6):691–693. [PubMed: 25551965]
- [53]. Smallwood RF, Laird AR, Ramage AE, Parkinson AL, Lewis J, Clauw DJ, Williams DA, Schmidt-Wilcke T, Farrell MJ, Eickhoff SB, Robin DA. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. *The journal of pain : official journal of the American Pain Society* 2013;14(7):663–675. [PubMed: 23685185]
- [54]. Smith SM, Nichols TE. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage* 2008;44:83–98. [PubMed: 18501637]
- [55]. Tatu K, Costa T, Nani A, Diano M, Quarta DG, Duca S, Apkarian AV, Fox PT, Cauda F. How do morphological alterations caused by chronic pain distribute across the brain? A meta-analytic co-alteration study. *NeuroImage Clinical* 2018;18:15–30. [PubMed: 30023166]
- [56]. Tessitore A, Russo A, Esposito F, Giordano A, Taglialatela G, De Micco R, Cirillo M, Conte F, D'Onofrio F, Cirillo S, Tedeschi G. Interictal cortical reorganization in episodic migraine without aura: an event-related fMRI study during parametric trigeminal nociceptive stimulation. *Neurological Sciences* 2011;32(Suppl 1):S165–S167. [PubMed: 21533737]
- [57]. Teutsch S, Herken W, Bingel U, Schoell E, May A. Changes in brain gray matter due to repetitive painful stimulation. *NeuroImage* 2008;42(2):845–849. [PubMed: 18582579]
- [58]. Tympanidis P, Terenghi G, Dowd P. Increased innervation of the vulval vestibule in patients with vulvodynia. *British Journal of Dermatology* 2003;148(5):1021–1027. [PubMed: 12786836]
- [59]. Vachon-Pressseau E, Roy M, Martel MO, Caron E, Marin MF, Chen J, Albouy G, Plante I, Sullivan MJ, Lupien SJ, Rainville P. The stress model of chronic pain: evidence from basal cortisol and hippocampal structure and function in humans. *Brain* 2013;136(Pt 3):815–827. [PubMed: 23436504]
- [60]. Ventolini G, Gyax SE, Adelson ME, Cool DR. Vulvodynia and fungal association: a preliminary report. *Med Hypotheses* 2013;81(2):228–230. [PubMed: 23707510]
- [61]. Wesselmann U, Bonham A, Foster D. Vulvodynia: Current state of the biological science, Vol. 155, 2014 pp. 1696–1701.
- [62]. Xia M, Wang J, He Y. BrainNet Viewer: A Network Visualization Tool for Human Brain Connectomics. *PLoS ONE* 2013;8(7):e68910–e68910. [PubMed: 23861951]
- [63]. Yager LM, Garcia AF, Wunsch AM, Ferguson SM. The Ins and Outs of the Striatum: Role in Drug Addiction. *Neuroscience* 2015;301:529–541. [PubMed: 26116518]
- [64]. Yen C-T, Lu P-L. Thalamus and pain. *Acta Anaesthesiologica Taiwanica* 2013;51:73–80. [PubMed: 23968658]
- [65]. Younger JW, Shen YF, Goddard G, Mackey SC. Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. *Pain* 2010;149(2):222–228. [PubMed: 20236763]
- [66]. Zatorre RJ, Fields RD, Johansen-Berg H. Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nature neuroscience* 2012;15(4):528–536. [PubMed: 22426254]
- [67]. Zatorre RJ, Fields RD, Johansen-Berg H. Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat Neurosci* 2012;15(4):528–536. [PubMed: 22426254]
- [68]. Zolnoun D, Bair E, Essick G, Gracely R, Goyal V, Maixner W. Reliability and Reproducibility of Novel Methodology for Assessment of Pressure Pain Sensitivity in Pelvis. *The Journal of Pain* 2012;13(9):910–920. [PubMed: 22958875]
- [69]. Zolnoun DA, Rohl J, Moore CG, Perinetti-Liebert C, Lamvu GM, Maixner W, Zolnoun D. Overlap Between Orofacial Pain And Vulvar Vestibulitis Syndrome. *Clin J Pain* 2008;24(3):187–191. [PubMed: 18287822]

Figure 1. ROI-Based Mask Based on Literature

PosCG: postcentral gyrus, SMA: supplementary motor area, PreCG: precentral gyrus, posINS: posterior insula, CaN: caudate nucleus, Put: putamen, NAcc: nucleus accumbens, Thal: thalamus, Hip: hippocampus. Color bar represents $1-p(\text{FDR})$ values.

Figure 2. Patients with provoked vestibulodynia (PVD, N=45) exhibit greater gray matter volume compared to healthy controls (N=45).

PreCG: precentral gyrus/motor cortex, PosCG: postcentral gyrus/somatosensory cortex, CaN: Caudate Nucleus, Hip: Hippocampus. Color bar represents 1-p(FDR) values.

Figure 3: Differences in gray matter volume comparing patients with provoked vestibulodynia (PVD; N=29) and patients with irritable bowel syndrome (IBS; N=29).
 pINS: posterior insula, PreCG: precentral gyrus Hip: hippocampus, THAL: thalamus, SMA: supplementary motor area, Put/Pal: putamen/pallidum.

Figure 4.
Summary of gray matter volume VBM Findings.

Figure 5.

Scatterplots for the partial correlations of gray matter with symptoms

Table 1:

Regions of Interest Based on the Destrieux/Harvard-Oxford Atlases Selected for Analyses

	Region	Atlas	Region Name from Atlas	Atlas Label	Reference
1	Posterior Insula	Nadich et al. 2004	N/A	pINS	2, 3, 6, 7, 8, 9, 10
2	Caudate Nucleus	Harvard-Oxford Subcortical	Caudate Nucleus	CaN	1, 2, 6, 7, 9
3	Globus Pallidus	Harvard-Oxford Subcortical	Pallidum	Pal	1, 2, 6, 7, 9
4	Hippocampus	Harvard-Oxford Subcortical	Hippocampus	Hip	5, 6, 7, 8, 10
5	Nucleus Accumbens	Harvard-Oxford Subcortical	Nucleus Accumbens	NAcc	1, 2, 6, 7, 9, 10
6	Putamen	Harvard-Oxford Subcortical	Putamen	Pu	1, 4, 6
7	Thalamus	Harvard-Oxford Subcortical	Thalamus	Tha	2, 3, 4, 6, 7, 10
8	Postcentral (Primary Somatosensory cortex/S1)	Destrieux	Postcentral gyrus	PosCG	2, 3, 5, 6, 9
		Destrieux	Postcentral Sulcus	PosCS	
9	Central Sulcus (Primary Somatosensory cortex/S1)	Destrieux	Central sulcus (Rolando's fissure)	CS	
10	Precentral (Primary Motor Cortex, M1)	Destrieux	Inferior part of the precentral sulcus	InfPrCS	2, 3, 9
		Destrieux	Precentral gyrus	PRCG	
		Destrieux	Superior part of the precentral sulcus	SupPrCs	
11	Secondary Somatosensory Cortex (S2)	Destrieux	Subcentral gyrus (central operculum) and sulci	SbCGS	3, 9, 10
	Supplementary Motor Area (SMA/Secondary Motor Cortex/M2)	Destrieux	BA6/ Superior Frontal Sulcus	SupFS	1, 5, 9, 10
12			BA6/ Superior Frontal Gyrus	SupFG	1, 5, 6, 9, 10

References Used to Obtain the Regions of Interest (ROI)

- ¹Gupta et al. (2015). Disease-related differences in resting-state networks: a comparison between localized provoked vulvodynia, irritable bowel syndrome, and healthy control subjects. [Pain 156](#).
- ²Bolwerk et al. (2013). Altered Resting-State Functional Connectivity in Complex Regional Pain Syndrome. [Pain 14](#) (10)
- ³Flodin et al. (2014). Fibromyalgia Is Associated with Decreased Connectivity Between Pain- and Sensorimotor Brain Areas. [Brain Connectivity 4](#) (8).
- ⁴As-Sanie et al. (2012). Changes in regional gray matter volume in women with chronic pelvic pain: A voxel-based morphometry study. [Pain 153](#).
- ⁵Bagarinao et al. (2014). Preliminary structural MRI based brain classification of chronic pelvic pain: A MAPP network study. [Pain 155](#).
- ⁶Labus et al. (2014). Irritable Bowel Syndrome in female patients is associated with alterations in structural brain networks. [Pain 155](#).
- ⁷Schweinhart et al. (2008). Increased gray matter density in young women with chronic vulvar pain. [Pain 140](#).

- ⁸Piche et al. (2013). Thicker Posterior Insula Is Associated With Disease Duration in Women with Irritable Bowel Syndrome (IBS) Whereas Thicker Orbitofrontal Cortex Predicts Reduced Pain Inhibition in Both IBS Patients and Controls. Pain **14** (10).
- ⁹Pukall et al. (2005). Neural correlates of painful genital touch in women with vulvar vestibulitis syndrome. Pain **115**.
- ¹⁰Seminowicz et al. (2010). Regional Gray Matter Density Changes in Brains of Patients With Irritable Bowel Syndrome. Gastroenterology **139**.
- ¹¹Farmer, M. A., Chanda, M. L., Parks, E. L., Baliki, M. N., Apkarian, A. V., & Schaeffer, A. J. (2011). Brain functional and anatomical changes in chronic prostatitis/chronic pelvic pain syndrome. *The Journal of urology*, *186*(1), 117-x124.
- ¹²Gupta et al (2018). Disease-Related Microstructural Differences in the Brain in Women with Provoked Vestibulodynia. Journal of Pain **(19)** 5.
- ¹³Naidich TP, Kang E, Fatterpekar GM, et al. The Insula: Anatomic Study and MR Imaging Display at 1.5 T. Am J Neuroradiol. 2004;**25**(2):222-232.

Table 2A:

Clinical Characteristics for the A. Vestibulodynia Subjects

Measurement	Range	Mean	SD	Sample Size
Gracely Differential Descriptor Pain Scale				
Pain Duration (months)	15-360	88.66	83.08	29
Level of pain intensity	0-13.5	5.90	4.84	29
Level of pain unpleasantness	0-14.5	5.29	4.35	29
Pain Related to Sex				
Level of Pain Related to Sex	0-100	57.43	26.05	29
Level of Pain Not Related to Sex	0-100	23.62	31.75	13
Vulvar Pain				
At 10 o'clock	0-9	3.32	2.78	45
At 7 o'clock	0-9	4.14	2.62	45
At 6 o'clock	0-10	4.49	2.41	45
At 5 o'clock	0-9	4.06	2.72	45
At 2 o'clock	0-9	3.41	2.56	45
Total Vulvar Pain Score	0-44	18.58	12.33	45
Vaginal Muscle Tenderness				
Bulba @ 5 o'clock	0-8	2.04	2.10	28
Bulba @ 7 o'clock	0-8	1.96	2.13	28
Pubococcygeus @ 6 o'clock	0-8	1.29	2.17	28
Levator @ 5 o'clock	0-7	1.82	1.93	28
Levator @ 7 o'clock	0-8	2.00	2.16	28
Total Vaginal Muscle Tenderness Score	0-31	9.11	8.87	28

Pain duration and level of pain intensity were assessed on a 21-point numeric rating scale (0=neutral, 20=extremely intense) using the Gracely Differential Descriptor Pain Scale. Level of pain unpleasantness was also assessed using a 21-point numerical rating scale (scale 0-20, 0=neutral, 20=very intolerable) in the past 24 hours using the Gracely Differential Descriptor Pain Scale. Level of pain related to sex and not related to sex were recorded on a scale of 0-100 (0 = no pain, 100 = most intense pain imaginable).

For vulvar pain, pain severity was measured on an 11-point Numerical Rating Scale where 0 = none and 10 = most severe pain imaginable and total vulvar pain was calculated by totaling the scores from the 5 sites (0-50).

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Vaginal muscle tenderness was measured on an 11-point Numerical Rating Scale, with 0 representing no pain and 10, representing the most severe pain imaginable, almost unconscious and total vaginal muscle tenderness score was calculated by totaling the scores from the 5 sites (0-50).

Abbreviations: PVD, provoked vestibulodynia; SD= standard deviation

Table 2B:

Clinical Characteristics for Irritable Bowel Syndrome Subjects

Measurement	Range	Mean	SD	Sample Size
<i>Bowel Symptom Questionnaire</i>				
Overall symptoms in the past week	1-17	9.14	3.96	29
Abdominal Pain in the past week	1-19	9.45	4.53	29
Bloating Pain in the past week	0-20	11.66	5.33	29
Usual Severity of Symptoms	2-5	3.31	.66	29
Age Onset	6-38	19.75	8.26	28
Duration of Symptoms (years)	0-30	8.72	6.71	25

Questionnaire: Overall symptoms in the past week were measured on a 0-20 scale (0=neutral, 20=extremely intense), Abdominal Pain in the past week was measured on a 0-20 scale (0=neutral, 20=very intolerable). Bloating Pain in the past week was measured on a 0-20 scale (0=neutral, 20=very intolerable).

Abbreviations: IBS, irritable bowel syndrome; SD, standard deviation

Significant disease related gray matter volumes differences between localized provoked vestibulodynia (PVD) patients and healthy control (HC) subjects

Table 3:

Cluster Index	Region	Cluster size	X	Y	Z	t	p (FDR)
CONTRAST: PVD (N = 45) > HC (N = 45)							
1	Right Caudate/ Putamen/ Nucleus Accumbens	375	18	26	2	3.15	.004
2	Right Hippocampus	386	24	-22	-14	3.44	.001
3	Left Caudate/ Nucleus Accumbens	156	-4	16	0	2.81	.002
4	Left Hippocampus	79	-16	-6	-24	2.59	.01
5	Left Postcentral Gyrus	89	-58	-22	48	2.75	.01
6	Right Postcentral Gyrus	49	48	-24	64	2.80	.01
7	Left Precentral Gyrus	23	-40	4	22	2.61	.02
8	Right Precentral Gyrus	11	36	-2	44	2.62	.02
CONTRAST: PVD (N = 45) < HC (N = 45)							
No Significance							

Contrast: PVD compared to HC

Groups: PVD, Provoked Vestibulodynia; HC: Healthy Control

Metric: t, t-statistic; p(FDR), FDR corrected p-value,

All regions represented in Montreal Neurological Institute (MNI) space with X, Y and Z coordinates.

Sig = p<0.05, FDR corrected

Significant disease related gray matter volume differences in vestibulodynia (PVD) patients compared to irritable bowel syndrome (IBS) patients

Table 4:

Cluster Index	Region	Voxels	X	Y	Z	t	p(FDR)
CONTRAST: PVD (N = 45) > IBS (N = 29)							
1	Right Hippocampus	369	32	-10	-26	4.34	2×10^{-4}
2	Left Hippocampus	133	-22	-16	-18	3.50	8×10^{-4}
3	Left Supplementary Motor Area	93	-40	20	20	3.52	.001
4	Left Precentral Gyrus	75	-38	4	24	3.91	2×10^{-4}
5	Right posINS	53	42	4	-8	3.85	4×10^{-4}
6	Left posINS	26	-34	-30	16	3.23	.009
7	Right Postcentral Gyrus	15	20	-38	78	2.79	.004
Contrast: IBS (N = 29) > PVD (N = 45)							
1	Left Thalamus/Putamen/Pallidum	3772	-34	-12	-8	5.64	2×10^{-4}
	Right Thalamus/Putamen/Pallidum						
2	Left Precentral Gyrus	7	-32	-20	42	3.97	0.002

Contrast: PVD compared to IBS

Groups: PVD, Provoked Vestibulodynia; IBS, Irritable Bowel Syndrome

Metric: t , t -statistic; p (FDR), FDR corrected p -value

All regions represented in Montreal Neurological Institute (MNI) space with X, Y and Z coordinates.

aINS (anterior insula), posINS (posterior insula)

Sig = $p < 0.05$, FDR corrected