Sex differences in brain modular organization in chronic pain

Camille Fauchon, David Meunier, Anton Rogachov, Kasey Hemington, Joshua Cheng, Rachael Bosma, Natalie Osborne, Junseok Kim, Peter Shih-Ping Hung, Robert Inman, et al.

Sex-differences in brain modular organization in chronic pain

Running title: Brain architecture, sex differences, chronic pain

Camille Fauchon[a,b], David Meunier[c], Anton Rogachov[a,b], Kasey S. Hemington[a,b], Joshua C. Cheng[a,b], Rachael L. Bosma[a], Natalie R. Osborne[a,b], Junseok A. Kim[a,b], Peter Shih-Ping Hung[a,b], Robert D. Inman[a,d], Karen D. Davis* [a,b,e]

[a] Krembil Brain Institute, Krembil Research Institute, University Health Network, Toronto, ON, Canada M5T 2S8;

[b] Institute of Medical Science, University of Toronto, Toronto, ON, Canada M5S 1A8

[c] Aix Marseille Univ, CNRS, INT, Inst Neurosci Timone, Marseille, France

[d]Division of Immunology, University of Toronto, Toronto, ON, Canada;

[e]Department of Surgery, University of Toronto, Toronto, ON, Canada;

*Corresponding author:

Karen D. Davis, PhD, FCAHS
Division of Brain, Imaging, and Behaviour, Krembil Brain Institute, Krembil Research Institute, University Health Network
399 Bathurst St, Room MP12-306
Toronto, ON M5T 2S8, Canada
Tel: (416) 603-5662
E-mail address: karen.davis@uhnresearch.ca

Number of pages (without tables): 43

Number of figures= 4; tables= 2 and 5 supplemental data tables

Number of words: abstract = 232, introduction = 500; discussion= 1498

Abstract

Men and women can exhibit different pain sensitivities and many chronic pain conditions are more prevalent in one sex. Although there is evidence of sex differences in the brain, it is not known whether there are sex differences in the organization of large-scale functional brain networks in chronic pain. Here, we used graph theory with modular analysis and machine-learning of resting-state (RS)-fMRI data from 220 participants; 155 healthy controls and 65 individuals with chronic low back pain due to ankylosing spondylitis (AS), a form of arthritis. We found an extensive overlap in the graph partitions with the major brain intrinsic systems (i.e., default mode, central, visual and sensorimotor modules), but also sex-specific network topological characteristics in healthy people and those with chronic pain. People with chronic pain exhibited higher cross-network connectivity, and sex-specific nodal graph properties changes (i.e., Hubs disruption), some of which were associated with the severity of the chronic pain condition. Females exhibited atypically higher functional segregation in the mid- and subgenual cingulate cortex and lower connectivity in the network with the default mode and fronto-parietal modules; whereas males exhibited stronger connectivity with the sensorimotor module. Classification models on nodal graph metrics could classify an individuals' sex and whether they have chronic pain with high accuracies (77-92%). These findings highlight the organizational abnormalities of RS-brain networks in people with chronic pain and provide a framework to consider sex-specific pain therapeutics.

Introduction

Brain structure is largely overlapping between sexes. However, there are specific morphological and functional brain differences between males and females [53,77] with certain features being more typical in one sex [42]. These brain differences may help explain a wide range of behaviour and clinical conditions that manifest differently in men and women [4,62], such as some aspects of acute and chronic pain [35,62]. Women generally have greater sensitivity to noxious stimuli [21], yet can exhibit greater heat pain adaptation and habituation than men to repeated, prolonged noxious stimuli [34]. The brain mechanisms underlying these sex differences are not known but emerging data has linked sex differences in pain sensitivity to resting state (RS) functional connectivity (FC) within the dynamic pain connectome (DPC)[13,14,35,39,46,47,68,78,79,86]. Brain architecture is reflected by intrinsically connected networks, whose regional activity is correlated during a RS [75]. The DPC concept emphasizes the flexibility of connections between RS-networks involved in the processing and modulation of noxious stimuli that shape pain perception including attention and salience processes [46]. We reported that women have stronger FC of the subgenual anterior cingulate cortex (sgACC) and areas of descending pain control system which could underlie their greater pain habituation [86]. In contrast, FC of the salience network (SN) was stronger in men, which could support greater sustained attention to pain [17,25,86].

Chronic pain conditions have been associated with widespread abnormalities in the activity and connectivity of RS-networks within the DPC, including, the SN, the default mode (DMN) and the sensorimotor (SMN) networks [2,3,11,14,19,39,44,45,47,56,80,82]. We have also shown

that the FC between the DMN and SN, is abnormally high in chronic pain [7,39]. Moreover, recent studies have identified sex differences in abnormalities found in some chronic pain conditions. For example sgACC abnormalities can be greater in women with chronic pain [32,68], whereas abnormalities in the SMN can be more prominent in men with chronic pain [79].

Despite our understanding of individual brain networks related to pain and the well-known sex prevalence of many chronic pain conditions [29,62], it is not known whether the organization of brain regions comprising large-scale human brain functional networks in patients with chronic pain exhibit sex differences. Isolating functional brain networks and examining their interactions can reveal the broader context of network abnormalities due to chronic pain in both sexes.

Thus, the main aim of this study was to characterize sex differences and abnormalities in the functional segregation and integration of the whole-brain network architecture associated with chronic pain. Towards this goal, we used graph theory with modular analysis [8,23,60], and machine-learning approach on RS functional magnetic resonance imaging (rsfMRI) data from healthy controls and individuals with chronic low back pain. We tested the hypotheses that: 1) there are sex differences in the modular organization of brain functional networks in healthy individuals; 2) chronic pain is associated with higher inter-networks connectivity and a sex-specific aberrant brain modular structure in networks of the DPC; and 3) graph-theoretical metrics can accurately classify individuals according to sex and the presence of chronic pain.

Materials and Methods

Participants.

The study consisted of data from 220 individuals: 65 with chronic low back pain (cLBP) due to ankylosing spondylitis (AS) (45 males; mean age ± SD = 34.5 ± 10.1, [19-61] and 20 females; mean age ± SD = 32.2 ± 10.3, [18-51]), and 90 age-sex matched HCs; 45 males mean age = 33.8 ± 10.0, [19-61]; 45 females mean age = 32.3 ± 10.3, [18-51]. In the statistical comparison analysis, we balanced the number of subjects in each group to avoid bias (45 male HCs vs 45 female HCs; 45 males with cLBP vs. 45 male HCs; and 20 females with cLBP vs. 20 female HCs). Another data sample from 65 males and females HCs controls (n=65; 31 males with a mean age ± SD = 30.8 ± 9.7, [18-54]; 34 females with a mean age = 33.1 ± 10.2, [18-62]) were included and used as reference to compare the graph partition (see below comparison of modular partition section). Participants were recruited from the community and provided informed consent to the study approved by the University Health Network Research Ethics Board. Patients with chronic pain were recruited from the Ankylosing Spondylitis clinic at Toronto Western Hospital. AS predominantly occurs in young males with relatively few co-morbidities. We excluded AS patients who were not experiencing pain as a primary symptom. The inclusion criteria for patients with chronic pain were: a diagnosis of AS based on the modified New York criteria, that includes low back pain unremitting with rest, and improved with exercise, impaired mobility of the spine and sacroiliitis finding at radiography [50]. The AS cohort reported an average (±SD) years living with pain of 14.7 (8.1) for males and 15.2 (10.8) for females, which ranged from 2 to 45 years. Although AS pain is classically associated with pathology localized to the sacroiliac joint (lower back), many patients reported bilateral pain radiating into their lower

extremities (e.g., knees and wrists). Twenty-nine males and eleven females of the patients were being treated with anti-TNF-α (biologics) medication (i.e., Enbrel, Humira, Remicade, and Simponi), and sixteen males and nine females were treated with non-steroidal anti-inflammatory drugs (i.e., Voltaren, Naprosyn, and Celebrex).8 AS patients (5 males) treated with biologics medication reported an average weekly pain close to 0-1 of 10, but had daily experiences of pain greater than 4/10. This was verified for all participants both patients and controls: (1) 18-65 years old, (2) the absence of acute pain or a history of chronic pain (other than AS in the patient group), (3) no prior diagnosis of neurological, psychiatric, or metabolic conditions (e.g., diabetes), (4) no major surgery in the 2 years or taking medications on a regular basis (except for the AS-related treatments) prior to study participation, and (5) no MRI standard contraindications. Inclusion criteria for the patients were stable medications, pain for >6 months, and absence of other major diseases. All subjects included in the study were right-handed.

fMRI data acquisition and pre-processing.

All study participants underwent a magnetic resonance imaging (3T GE) session to acquire a high-resolution T1-weighted anatomical scan (1 × 1 × 1-mm3 voxels, matrix = 256 × 256, 180 axial slices, repetition time = 7.8 s, echo time = 3 ms, inversion time = 450 ms) and a T2*-weighted resting-state functional magnetic resonance imaging scan (3.125 × 3.125 × 4-mm3 voxels, matrix = 64 × 64, 36 axial slices, repetition time = 2 s, echo time = 30 ms, flip angle = 85°, 277 volumes, total scan time = 9 min, 14 s). For the resting-state scan, participants were instructed to “close your eyes; do not try to think about anything in particular; do not fall asleep.”

The preprocessing of the resting-state functional magnetic resonance imaging (rsfMRI) data was performed using the FEAT (functional magnetic resonance imaging expert analysis tool) toolbox in Oxford Centre of Functional Magnetic Resonance Imaging of the Brain's (FMRIB's) Software Library (FSL). The first four volumes of the rsfMRI scan were removed, nonbrain tissues were extracted using the Brain Extraction Tool (BET) function, and motion correction was performed using Motion Correction FMRIB's Linear Image Registration Tool (MCFLIRT). OptiBET was used to strip the skull of the T1-weighted anatomical images [54]. Next, each participant's functional images were registered to the skull stripped, T1-weighted anatomical images using FLIRT, followed by nonlinear registration to MNI152-2 mm space using FMRIB's Non-linear Image Registration Tool (FNIRT). aCompCor was used to remove scanner-related and physiological noise [12]. Non-brain volumes (i.e., Cerebrospinal fluid and white matter), and 6 motion parameters were regressed out to only keep the grey matter. High-pass (0.01 Hz cutoff) temporal filter was applied to remove intrinsic scanner-related signal drift.

Graph theory analysis

Brain graph construction.

Graph analysis was performed using the graphpype functions of the open-source neuropycon package [61] (https://neuropycon.github.io/graphpype/), which is based on Nipype [28]. It provides a formal characterization of the brain connectome organization [22]. Individual graphs (Fig. 1) were formed using the 360 cortical and subcortical nodes (i.e., 180 brain regions by hemisphere) of the Human Connectome Project brain atlas (cerebellar regions were not included) and edges were defined using functional connectivity between nodes (i.e., synchrony of activity between brain regions). Raw time-series data were averaged and extracted over the voxels within

the HCP nodes. Correlation matrices were computed individually by computing Pearson correlations between the time courses of every pair of nodes (360 x 360) from the preprocessed rsfMRI data [1,58]. The correlation values were converted to Z-score with Fischer's transform for subsequent statistical analysis. The number of possible links in a network of 360 nodes is equal to 359*360/2=64620.

Such networks are referred to as fully connected since there is a nonzero link between every pair of regions. However, the brain is not a fully connected network and all these connections are not functionally relevant and include a percentage of spurious connections (reflecting a measurement of noise rather than the presence of an actual connection). To address this problem and keep the sparsity aspect of a graph, network density thresholds [49] were applied to eliminate weak edges (i.e. reduce noise and variability between each connectivity matrices). The threshold level is a parameter which influences network properties (because correlations under that threshold were ignored); Low thresholds, with high connection densities generate graphs with low modularity equivalent to a random graph, whereas high threshold could generate disconnected graphs in which some regions were not linked to any other brain region. Thus brain networks were constructed and analyzed over a range of thresholds designed to focus on fully-connected but non-random aspects of brain network organization [1,56,58,71] beginning at 1% to 10% link density (i.e., retaining the top 1% to 10% of correlations). The graph illustrations presented are for 5%, corresponding to 3231 links (thresholded at a sparse connection density equivalent to ~5% of 64620).

We also constructed an average Z-correlation matrix for each group by averaging the connectivity matrix of subjects after thresholding to depict the whole brain network structure associated with male and female HCs and those with chronic pain. The visualization of modules and anatomical nodes on a brain surface was created using the open-source python software visbrain [15].

Network topological features.

The topological properties of graph networks were characterized by computing the following graph metrics [22,65,87]: i) Clustering coefficient is a measure of information segregation reflecting the density of connections; ii) whereas global efficiency is a measure of information integration, it represents how well a network can transmit information at a global level; iii) Assortativity is a measure of connectedness between nodes [64]. A positive correlation indicates that the nodes with similar degree tend to attach together (assortative network) whereas negative correlation means than node with different degrees tends to attached together (disassortative network); iv) For each subject, we also computed the small-worldness coefficient, that uses a ratio of network clustering and path length compared to its random network equivalent. Graph networks have 'small-world' properties if the ratio is > 1 [$(C/C_{random}) / (L/L_{random})$], reflecting a good trade-off between network segregation and integration [41].

Modularity analysis.

A modular analysis was applied to study whether resting state functional brain activity is organized into modules. Modular decomposition (or community detection) of a graph aims at deciphering is there are modules (or subgraphs) whose nodes interact more strongly together

than they do with other nodes in the network [23,24,66]. The term “network” is used in many ways. In the present work, we used the term “module” defined as a set of nodes that have many intra-modular connections (i.e., highly interconnected) but sparser inter-modular connections with other nodes in the brain network. The modularity value indicates the level of decomposability of the system into smaller subsystems [9]. Several algorithms have been proposed to detect these modules [67] . Here, the modular analysis, and all aforementioned and hereafter graph measures, were estimated using the Radatools software [27] based on the individual connectivity matrix (http://deim.urv.cat/~sergio.gomez/radatools.php). This software allows for fine tuning of the optimisation for modular partitions, here in our workflow the modularity detection was optimized across 100 iterations.

Comparison of modular partitions.

We determined first whether there are sex differences in the brain modular organization in HCs. Second, to better understand the atypical brain organization in patients with cLBP, we measured the degree of modular structure similarity. The similarity between two sets of node partitions was quantified using normalized mutual information (NMI), which measures how much information one set of assignments provides about another set of partitions [18]. If the two partitions are identical, the NMI value is maximum and equal to 1. If the partitions are totally independent, the NMI value is null. The similarity of modular structures in each group (i.e., between males and females HCs and those with chronic pain) were assessed using NMI in relation to the group average of another independent sample of males and females healthy controls, not used so far in the study (n=65; 31 males and 34 females) to ensure that outcomes were unbiased (as previously used in [56,59]). First, we generated a baseline modular structure from this other set of HCs

using the same pipeline of analysis. Consistently, the control resting state functional network was segregated into 6 modules corresponding to commonly observed subnetworks (including DM, SM, central, visual, temporal and subcortical modules). The “central” module, previously defined in [58,59], clustered nodes of the salience system, and some somatosensory-motor nodes (i.e., cingulo-opercular cortex). The NMI score was computed for each group using the radatools software in relation to this previous partition arising from the second group of HCs.

Nodal graph properties associated with sex differences and chronic pain.

Connectivity is not distributed uniformly across the nodes that point the existence of highly connected brain hubs in resting state functional networks that may be influenced by sex differences and chronic pain. Hubs play central roles by integrating and distributing information in powerful ways due to the number and positioning of their contacts in a network [72]. The topological role of each node was determined based on its density of intra- and inter-modular connections, by tracking respectively, the within-module degree (WMD) and the participation coefficient (PC) of each node [30,31,58].

The WMD shows how well nodes are connected within modules; it measures the number of links of node compared to other nodes of the same module. The PC measures the distribution of node’s links among the modules. The PC is close to one if the node is extensively linked to all other modules and zero if it is linked exclusively to nodes of its own module. The nodes were classified into four categories according to the following criteria [58,81]: If PC > 0.3 (i.e., mean PC + 1 SD) the node was classified as a satellite connector. If WMD > 1.0, the node was defined as a provincial hub. If PC > 0.3 and WMD > 1.0, the node is classified as connector Hub;

otherwise, it is defined as peripheral nodes. The topological role of each module can be defined and compared in terms of the proportion of connector Hubs and provincial Hubs it contains (Fig. 1*A*). The connectors coefficient (i.e., percentage of connector Hubs + satellite connectors) and Hubs coefficient (percentage of connector Hubs + provincial Hubs) were also computed to describe the network topological structure.

A measure of PC alone can hide some shifts in connectivity, thus we also examined small changes in node modular association, which may influence the profile of interconnectivity between modules. For that, we computed a complimentary measure the Node Dissociation Index (NDI; proposed and assessed by [10]). Increased NDI would represent a node less associated with its own module and more associated with outside modules. In order to quantify the dissociation property for a module, the module dissociation index (MDI) is calculated as the mean NDI for all nodes in one module [10]. The MDI is calculated for each module in each group, which allowed the statistical comparisons of modules in common between HCs group and chronic pain group both in males and females.

Statistical analysis

First, to assess the significance of global topological properties (i.e., modularity, global-efficiency, etc.) and nodal graph metrics in each group (i.e., PC and WMD), we compared them to random networks that share the size and number of links of the graph network using the same analysis pipeline and permutation tests. Permutation tests were applied by first shuffling the values in the connectivity matrix 5000 times, and then computing pipeline on the shuffled matrix. This test was used because of the expected non-normal distribution of differences in

network measures. This procedure was repeated in the four groups of subjects (i.e., male and female with cLBP or HCs).

Second, significant group effects on graph properties associated with sex differences and/or chronic pain, were evaluated using permutations tests again, by shuffling (5000 times) the group belonging of the subjects, and comparing the original difference between groups to the distribution of differences after permutation. Post hoc Tukey-honestly significant difference were performed in case of a significant group effect. False discovery rate (FDR) correction was applied to avoid false results due to pure chance.

Third, NMI scores, and the density of connections within- and between the modules were entered in two-way ANCOVAs with sex and chronic pain as factors and age as covariates of non-interest. Pairwise group differences between groups were determined using Tukey post-hoc test. Group specific changes in MDI were determined using Mann-Whitney test (corrected for age and multiple comparisons). Effect sizes were computed with the cohen's d and the confidence intervals were bias-correlated and accelerated. These statistics and estimation plots were achieved under the estimation stats package built in Python [40] (https://github.com/ACCLAB/DABEST-python or https://www.estimationstats.com to access the user interface).

SVM classification based on nodal graph metrics.

Support vector machines (SVM) is a method for supervised classification that learns the relationship between a set of input features (i.e., nodal graph metrics) and a particular outcome

(i.e., the sex or the chronic pain of the subject) across a set of observations [16]. Given the sex-specific graph modular characteristics in chronic pain and healthy individuals**,** we investigated whether brain graph properties carried enough information to classify participants based on the presence of chronic pain and sex. However, an extensive overlap with no clear-cut also exists between the brain organization of males and females. To this end, machine-learning methods are appropriate. Specifically, SVM technique using a linear kernel was employed to train models for classification of the subject's sex or chronic pain from nodal graph metrics of PC and WMD (i.e., 360 features by model = number of nodes). Graph features based on whole-brain connectivity are of extremely high dimensionality. We therefore performed dimensionality reduction using a mass univariate approach which leveraged ANOVA F-statistics on each feature and incorporated within the cross-validation procedure, as previously described [92]. This does not refer to the F-score, also known as F-measure, which is a performance metric that is calculated from precision and recall and represents how well a given model worked [73]. Rather, here we performed mass univariate ANOVA then selected features that had top 5% F statistics in a cross-validated fashion for our model. The features were selected within each training fold only, separate from the testing fold to provide an estimate of out-of-sample model performance. Thus, the training and test set are independent, and all feature selection was performed only on the training set. For each model, the classifier was trained with either sex differences labels (male and female) or chronic pain labels (cLBP or HCs) as the outcome variable. Model out-of-sample generalizability was estimated through 5-fold cross validation. Classification significance was assessed using permutation approach (i.e. how unlikely the results would be if the classifier was randomly attributing the class labels), we repeated the entire

classification procedure (including the validation models, parameter optimisation, and feature selection) 1,000 times, each time permuting the labels.

Identifying graph metrics associated with clinical score: BASDAI.

We conducted two analyses to determine which graph metrics are associated to the clinical score BASDAI [26] (Bath Ankylosing Spondylitis Disease Index) at the global and nodal level. The BASDAI (0-10 scale, 10 = high disease activity) provides a comprehensive summary of symptom severity, including fatigue, spinal pain, joint pain and swelling, localized tenderness, and morning stiffness.

First, we used linear regression model to analyse the relationship between BASDAI score and global graph metrics (i.e., modularity, assortativity, global-efficiency, clustering-coefficient) adjusted for the potential effect of age and corrected for multiple comparison (FDR). We also examined the relation of global graph metrics with the McGill pain questionnaire [57].
Second, we used the support vector regression (SVR) algorithm to predict the BASDAI score based on the within-module degree value of nodes separately in males and females with chronic pain groups. SVR is a variant of SVM that provides a continuous outcome variable, in this case, the predicted BASDAI score. Machine-learning approaches can consider multiple-comparison issues, which is relevant in large-brain network, to identify the set of features (i.e., nodes in the network) associated with a non-brain variable. Briefly, a SVR searches for a function that describes the relationship between multivariate input with the continuous outcome score while minimizing the error. Following the same feature selection process as in SVM experiments which retains F-statistics-based top 5% of features, the SVR procedure with a linear kernel (age

corrected) was applied on WMD data to predict BASDAI scores in males and females with chronic pain. Model generalizability was also estimated through 5-fold cross validation.

Results

Our findings addressed 3 main research questions:

1) Are there sex differences in the modular organization of brain functional networks in healthy individuals?

2) Is chronic pain associated with a sex-specific aberrant brain modular structure?

3) Can graph theoretical metrics accurately classify individuals according to sex and the presence of chronic pain?

Common global network topological features across sexes in health and chronic pain.

We examined the brain graph topological features of 65 individuals (45 males, 20 females) with chronic low back pain (cLBP) due to ankylosing spondylitis, and age-sex matched healthy controls (HCs).

We found that brain functional networks were consistently modular in each group and over a range of density thresholds (Fig. 1*B*). The maximum modularity declined monotonically as a function of increasing connection density (Fig. 1*B*). The global network metrics computed (i.e., modularity, clustering coefficient, global-efficiency) were significantly different compared to random graphs ($p<0.001$), but there were no significant differences associated with sex or chronic pain (see Supplemental Table 1). All groups exhibited the typical features of small world organization (mean±SD =1.63±0.17) [87]. The modularity stabilized at 5% link density in all

groups, corresponding to 3231 links (i.e., thresholded at a sparse connection density equivalent to ~5% of 64620 in a network of 360 nodes). Thus, we focused our subsequent analysis on brain graphs constructed at 5% since this produces sparse graphs with consistent features [8,56,58].

Sex differences in brain modular organization in healthy individuals.

The brain network in the healthy males and females (n=45 in each group) were segregated into 6 consistent and connected modules corresponding to commonly observed large-scale resting state networks [1,56,71]. We found four common modules and two distinct modules between both sexes (Table 1). The modules that were common to both healthy males and females were part of the following systems: 1) default mode (DM): precuneus, angular gyrus and medial prefrontal cortex, 2) visual: calcarine sulcus, cuneus and entorhinal cortex, 3) central: insula, mid cingulate cortex (MCC), and cingulo-orpercular cortex [58], 4) sensorimotor (SM): supplementary motor area (SMA), primary somatosensory (S1) and motor cortex (M1), parietal superior. The distinct functional segregations in healthy males included a temporal module (association and lateral temporal areas) and a ventromedial prefrontal cortex (vmPFC) module. In the healthy females, the distinct modules were a frontoparietal (FP) module (dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), inferior frontal and precuneus) and a subcortical module (entorhinal cortex and hippocampus).

Identification of Hubs in healthy men and women.

Given the distinct sex-specific modular patterns of organization we identified in healthy individuals, we next asked whether there are sex differences in the Hubs location and distribution (Fig. 1*A*). Nodes were classified in four categories based on their intra- and inter-modular connectivity (see Methods).

The global number (mean ±SD) of connectors Hubs were higher in females (30.2 ±6.2) than in males (21.8 ±8.3; $t_{(88)}$=-5.7, p<0.001), whereas the number of provincial Hubs was similar in both sexes ($t_{(88)}$=-1.8, p=0.07). Specifically, in healthy males and females, we identified 40.4(9.9) and 36.3(10.1) provincial Hubs; 109.8(9.2) and 115.2(9.9) satellite connectors; 198.4(8.1) and 178.6(9.4) peripheral nodes. The location of most of the connectors Hubs in males were located in the temporal, DM and vmPFC modules, whereas in the females they were in the FP, SM, DM and subcortical modules. In contrast, provincial Hubs were distributed well in both sexes among modules (see Supplemental Table 2). Sex-specific nodal graph metric changes in HCs assessed by permutation test were mainly found for the WMD and PC in the DM (e.g., PCC, OFC, ACC)), central (e.g., inferior parietal cortices (IPC) and inferior frontal gyrus (IFG)), temporal and SM (e.g., premotor nodes, SMA) modules (details in Supplemental Table 3).

Chronic pain is associated with sex-specific abnormalities in brain modular architecture. We first compared the modular partitions in patients with chronic pain (45 males, 20 females) to those in age and sex matched-healthy controls (Fig. 2, 2A). We found that the modular segregation on average was the same in males with cLBP compared to healthy males, at 5% link density the same six modules were found (Fig. 2B). In contrast, compared to healthy females, the females with chronic pain exhibited a modular organization comprised of 8 modules (Fig. 2C and pie plots). The nodes associated with the DM module were grouped into two smaller modules in females with cLBP: an MCC module and a small subgenual anterior cingulate cortex (sgACC) module. Although the average number of modules was almost similar between the HCs

and cLBP groups, the individual modular organization including nodes membership was perturbed in the patients and differed between males and females.

Second, we quantified the changes in modular architecture associated with chronic pain and sex differences by computing the normalized mutual information (NMI) in relation to the baseline modular structure from separate sample of HCs (n= 65). The NMI plots in Fig. 2A showed significant changes according to chronic pain ($F_{(1,126)} = 6.35$; $p = 0.03$) and sex ($F_{(1,126)} = 4.69$; $p = 0.03$) but without significant interaction ($F_{(2,126)} = 0.11$; $p = 0.74$). We observed that males and females with chronic pain showed significantly lower NMI than the healthy males ($p<0.001$) compared to the independent healthy group. In addition, the NMI values for females were lower compared to males in both healthy and chronic pain groups ($p< 0.016$).

Third, we next tested the impact of sex and chronic pain on the global within- and between-modules connectivity (Fig. 2A). There was no overall effect of the sex or chronic pain factors on the density of within-module connections, but there is an interaction ($F_{(2,126)} = 4.82$, $p = 0.03$). In chronic pain group, the within-module degree density was lower in males, but higher in females compared to HCs. There was no effect of sex on the total number of between-module connections and no interaction but, the number of between-module connections was greater in those with chronic pain compared to healthy controls ($F_{(1,126)} = 4.90$, $p = 0.02$).

At the nodal level, in males with cLBP compared to male HCs: several areas had significantly greater PC including nodes of the SM module, subcortical and prefrontal nodes, while the PC was lower in SMA and M/ACC nodes; The WMD was greater in the temporal module, op.

frontal, IPC and MCC nodes, while the WMD was lower in SM module and mPFC, DLPFC nodes.

In females with cLBP, the PC and the WMD were significantly higher in a group of nodes associated with attentional or salience processing (i.e., anterior insula, TPJ, M/ACC, IPC) and vmPFC and were lower in the SM module, operculo-insular, PCC, IFG, DLPFC nodes (see Supplemental Table 3).

Fourth, to investigate the possibility that brain abnormalities in chronic pain involve global Hub disruptions, we classified the nodes according to the four previous categories. Respectively in males and females with cLBP, we identified (mean±SD): 26.4(9.1) and 34.7(9.8) nodes as connector Hubs; 33.6(13.8) and 25.2(11.9) as provincial Hubs; 118.9(9.7) and 126.2(10.3) nodes as satellite connectors; 182.4(9.5) and 173.9(10.2) as peripheral nodes (Fig. 2B, 2C radar charts).

The connectors and Hubs coefficients were: 40% and 16.7% in males with cLBP against 33.8% and 17.2% in males HCs, 44.7% and 16.7% in females with cLBP against 40.3% and 18.3% in HCs females (extended data table Figure 2-2). We found a greater connector coefficient in males with cLBP in the DM, temporal and central modules compared to healthy males (i.e., cLBP vs. HCs: 40.4% and 31.9%; 24.6% and 18.3%; 36.1% and 23.9%). In female with cLBP, the connector coefficient was also higher in most of modules compared to healthy females. Patients with cLBP had globally a higher proportion of connector Hubs corresponding to more connections between the modules compared to HCs in both sexes ($F_{(1,126)} = 4.12$; $p = 0.035$). In

females with chronic pain, the MCC module showed a high level of connections with other modules (i.e., connector coef. = 94.4%, see Supplemental Table 2).

Chronic pain is associated with sex-specific modular connectivity profile disturbances.

We examined the changes in modules connectivity profile associated with chronic pain via module dissociation index (MDI). In both sexes, patients with cLBP had atypical connectivity in some cortical modules compared to the HCs (Fig. 3). In males with cLBP, the MDI was significantly higher in the SM module compared to healthy males (Cohen's d = 0.40 [95.0%CI 0.031, 0.77], $p = 0.016$). In females with cLBP, the MDI was lower in the FP (Cohen's d = -0.38 [95.0%CI -0.71, -0.03]; $p = 0.013$) and the DM module (Cohen's d = -0.43 [95.0%CI -0.73, -0.11]; $p = 0.012$) compared to healthy females.

Support vector machine classification performance.

Out of all of graph metric-based SVM models, WMD models provided the best prediction accuracy for the classification between sexes and the presence of chronic pain (Table 2 and Supplemental Table 4). The SVM models derived from nodal WMD can classify individuals based on their sex and presence of chronic pain with excellent accuracy ranging from 77% to 92% ($p < 0.001$). Males and females are separated with an accuracy of 81% in healthy group (i.e., nodes located in the PCC, DLPFC, ACC, TPJ, parietal operculum, temporal cortex); and with an accuracy of 92% in chronic pain (i.e., nodes located in the DLPFC, parietal, subcortical, TPJ, gyrus frontal inferior). Females with chronic pain and HCs were classified with an accuracy of 85% (i.e., nodes in the vmPFC, DLPFC, S1, superior parietal, visual, lateral temporal

cortices). Males with chronic pain and HCs were classified with an accuracy of 77% (i.e., nodes in the DLPFC, OFC; ACC, frontal operculum).

Graph metrics associated with clinical scores.

Given the broad changes of graph organization in chronic pain, we hypothesised that some global graph properties are associated with pain (McGill pain questionnaire) and severity of AS in terms of function (i.e. BASDAI score).

We found a significant negative relationship between BASDAI scores and assortativity value (p = 0.017; R = -0.36) in males with cLBP. The network global efficiency was also associated with the pain scores (p=0.032; R= -0.32) in males with cLBP (Fig. 4A). No significant relationships were found with modularity values or other global graph metrics. In addition, we did not find any significant relationships between these variables in females with cLBP (corrected for age and multiple comparison (FDR)).

We next investigated whether the WMD of some nodes in the network correlated significantly with BASDAI. The SVR model showed that the WMD were associated with BASDAI (r^2 = 0.45) in males with cLBP, including nodes in the temporal (STG), the DM (PCC, medial prefrontal cortex, OFC), the vmPFC modules and nodes in the TPJ, S1, premotor cortex, DLPFC and ACC. In females with cLBP, the SVR analysis showed that the WMD were associated with BASDAI (r^2 = 0.54), including mainly nodes in the DM and subcortical modules and some nodes in the visual, central, SM, FP (i.e., DLPFC, vmPFC and temporal cortex) modules (Fig. 4B and Supplemental Table 5).

Discussion

This study provides novel insight into sex-specific functional organization of resting-state brain networks in healthy individuals and sex-related abnormalities in people with chronic pain. Our key findings (Fig. 5) indicate that: 1) An extensive overlap exists between the modular partitions of healthy men and women, but some graph features are more common to each sex. 2) People with chronic pain exhibit higher cross-network connectivity, but only the women have atypical modular segregation in the MCC and sgACC compared to HCs. 3) The connectivity profile of men with chronic pain is stronger in the SM module, whereas women with chronic pain have lower distributions of connections with the DM and FP modules. 4) People with chronic pain exhibit sex-specific nodal graph properties changes (i.e., Hubs disruption), some of which were associated with the severity of their condition. 5) Graph metrics can be used to classify an individuals' sex and whether they have chronic pain with high accuracies. Therefore, we have demonstrated that sex differences exist in the organizational abnormalities of RS-brain networks in people with chronic pain, incorporate sex as a variable will improve future development of individual targeted pain management therapeutics.

Based on previous findings of sex differences in RS-functional connectivity [77,88,90], we predicted that the brain modular organization in HCs would be different in males and females. In our first analysis, we found distinct functional segregations of nodes located for males in the temporal cortex and the vmPFC, and for females in fronto-parietal and subcortical cortices (Fig. 2 and Fig. 5). Females exhibited stronger inter-connectivity than males in the PCC, vmPFC, but

weaker connectivity in the ACC, and superior temporal gyrus (also shown in [5]). These findings are in line with a recent study [88] showing that brain regions with the highest sex classification accuracies were mainly located in the cingulate cortex (i.e., ACC and PCC), prefrontal cortex, temporoparietal regions and precuneus. Interestingly, in our results, there were sex differences in functional connectivity (i.e., PC and WMD) of the DMN; findings in this network of interest has also been reported in several large cohort studies comparing male's and female's brain [5,77,88,91]. Additionally, our finding of more connectors regions in females, suggests that there is more brain integration in females than in males (similar to [90]). We previously found that compared to men, women have stronger sgACC FC with nodes of the pain modulation system [86], and proposed that this may explain why women have greater attenuation and habituation to sustained, repeated pain stimuli [34,35]. Taken together, although we did not directly measure pain sensitivity or habituation, our results point to stronger communication within and between fronto-parietal structures in females as a mechanism to facilitate engagement of the descending antinociceptive system and produce greater pain modulation efficacy compared to males. The higher functional segregation of nodes in the vmPFC in males may reflect weaker engagement of the antinociceptive pathway, resulting in reduced pain habituation [86].

Chronic pain may alter the integrity of brain resting-state networks [1,2,36,43,46,51,55,56,82]. Our finding that chronic pain patients of both sexes had lower similarity values indicates that the modular partitions were less homogeneous in chronic pain than is normally the case (Fig. 2*A*). Additionally, the dissimilarity of individual graph partitions was more pronounced in female patients which represented modular patterns more diffuse than in controls and in males with chronic pain. Thus, global graph abnormalities in the brain functional organization are primarily

driven by female patients, as also observed in [79]. Concurrently, compared to controls, males with cLBP showed an equivalent modular structure, whereas female patients had greater modular segregation in RS-networks as has been observed before in healthy and chronic pain patient populations [36,83].

Previously, we and others demonstrated functional changes in the dynamic pain connectome, and proposed that three specific networks (i.e., DMN, SN, SMN) play a prominent role in chronic pain, including an hyper-connectivity between DM – SM networks [6,13,38,39,52,79]. Here, males and females with chronic pain exhibited higher inter-modular connectivity in the whole brain network at rest (Fig. 5). The patients with high BASDAI and pain scores had a brain structure less efficient for information flow and disassortative (more vulnerable [65]), and associated with a widespread pattern of nodal changes.

The nodes comprising DM and FP modules were markedly variable in women with chronic pain, showing a significantly lower distribution of connections with other modules and were more connected their own module (i.e., reduced MDI; Fig. 4) compared to with the female HCs. Disruption of the activity and connectivity of DMN occurs across multiple chronic pain populations [2,52,63,79]. Our female patients also exhibited heightened level of intra-functional connectivity between nodes of the MCC, an area associated with pain affect [76,85], attentional tasks, motor reaction and alert [20,70]. The MCC module is fully integrated in the network, showing a large proportion of connector Hubs. We also found in female patients that nodes located in the sgACC, a core structure of the pain modulatory system, interact strongly together in one distinct module and lower with other nodes in the brain network. In line with this, earlier

sex-difference studies suggested women with chronic pain may have a greater responses and altered connectivity in sgACC compared to men [32,48,68]. This may indicate a shift of information away from DMN to attentional/cognitive FP network, implying a stronger integration of regions typically associated with pain affect, attentional processes and lower connections with regions involved in descending pain controls such as the sgACC. In accordance with this speculative interpretation, in female patients the BASDAI scores were associated with the connectivity of DM nodes, and the number of Hubs in high-order networks, underlying the capacity to transmit information, is higher compared to HCs.

Our findings also suggest that SM cortex nodes are more inclined to form pairwise links with other nodes and modules in the brain network of males with cLBP. Recent reports have revealed extensive reorganization of the SM network [55] and altered hub topology [43] in chronic pain. The sensorimotor network has been consistently implicated in chronic pain [89], and widely used as a target for brain stimulation [69,74,84]. Here, the SM abnormalities are mainly associated with male patients. This is consistent with previous studies showing higher BOLD variability in SM network at high-frequencies in male patients compared to female patients with chronic pain [79]. This abnormal distribution of connections in the SM module could be due to cortical plasticity following a lengthy period of nociceptive inputs. Whereas the neurophysiological significance behind these discrepancies is unclear, it may suggest sex-specific sensory-motor processing in chronic pain patients.

As depicted in Fig. 5, we propose that people with chronic pain generally exhibit abnormally high resting state cross-network interactions within the DPC. Since chronic pain impacts many

aspects of brain functions, the existence of widespread connectivity changes was anticipated [2,39,55,82]. However, here we also showed sex differences in the abnormalities of brain functional organization in people with chronic pain; that is atypical modular segregation, and FP / DM connectivity changes in females and higher connectivity of SM in males. An important distinction though must be made between these sex differences in a chronic pain state and sex differences that are intrinsically present in a healthy state. Accordingly, the classification models of patients and controls according to sex performed very well and the accuracy (77-92%) was comparable with that seen in other machine learning-based studies [55]. This showed that there are sufficient brain topological changes in chronic pain and between both sexes to allow reliable classification. These findings may account for the sex differences in pain modulation and the prevalence of chronic pain.

There are some methodological limitations for future considerations. Given our modest sample size, univariate dimensionality reduction was performed using ANOVA F-statistics within a cross-validation scheme. Cross-validation procedure can provide an adequate estimate of out-of-sample model performance, but there is value in validating against external datasets. Future studies can evaluate model performance in independent external validation cohorts. The effects of a specific brain parcellation and modular resolution parameters [37] may influence the network topology. Sex-differences and chronic pain abnormalities may be encapsulated at different modularity scales. Future studies should also examine whether the current findings are generalizable across multiple chronic pain conditions. [60].

In conclusion, our data support the notion of chronic pain as a network disorder occurring within the dynamic pain connectome that include some sex-specific abnormalities of the brain functional organization. Compared to brain organization in healthy people, the chronic pain brain is more integrated, reflected by a substantial increase of cross-network communication and connector Hubs. These changes in baseline connectivity that develop with chronic pain may participate to the maintenance of an hypersensitivity. Altogether, the present results demonstrate that studies of chronic pain that do not examine data in both sexes separately, risk bias in interpreting the findings, are at risk to miss important information, and in the case of single sex studies, may have limited generalizability [33]. Thus, our findings provide a framework to understand sex differences in the brain that may reflect chronic pain.

Conflicts of Interest and Acknowledgements

The authors declare no competing interest.

This work was supported by the Canadian Institute of Health Research (PJT 156409, and SCA-145102 Strategy for Patient-Oriented Research (SPOR) funding of the Canadian Chronic Pain Network) and The Mayday Fund.

References

[1] Balenzuela P, Chernomoretz A, Fraiman D, Cifre I, Sitges C, Montoya P, Chialvo DR. Modular Organization of Brain Resting State Networks in Chronic Back Pain Patients. Frontiers in Neuroinformatics 2010;4.

[2] Baliki MN, Geha PY, Apkarian AV, Chialvo DR. Beyond Feeling: Chronic Pain Hurts the Brain, Disrupting the Default-Mode Network Dynamics. Journal of Neuroscience 2008;28(6):1398-1403.

[3] Baliki MN, Mansour AR, Baria AT, Apkarian AV. Functional Reorganization of the Default Mode Network across Chronic Pain Conditions. PLoS ONE 2014;9(9):e106133.

[4] Berkley KJ. Sex differences in pain. Behavioral and Brain Sciences 1997;20(3):371-380.

[5] Biswal BB, Mennes M, Zuo XN, Gohel S, Kelly C, Smith SM, Beckmann CF, Adelstein JS, Buckner RL, Colcombe S, Dogonowski AM, Ernst M, Fair D, Hampson M, Hoptman MJ, Hyde JS, Kiviniemi VJ, Kotter R, Li SJ, Lin CP, Lowe MJ, Mackay C, Madden DJ, Madsen KH, Margulies DS, Mayberg HS, McMahon K, Monk CS, Mostofsky SH, Nagel BJ, Pekar JJ, Peltier SJ, Petersen SE, Riedl V, Rombouts SARB, Rypma B, Schlaggar BL, Schmidt S, Seidler RD, Siegle GJ, Sorg C, Teng GJ, Veijola J, Villringer A, Walter M, Wang L, Weng XC, Whitfield-Gabrieli S, Williamson P, Windischberger C, Zang YF, Zhang HY, Castellanos FX, Milham MP. Toward discovery science of human brain function. Proceedings of the National Academy of Sciences 2010;107(10):4734-4739.

[6] Bosma RL, Cheng JC, Rogachov A, Kim JA, Hemington KS, Osborne NR, Venkat Raghavan L, Bhatia A, Davis KD. Brain Dynamics and Temporal Summation of Pain Predicts Neuropathic Pain Relief from Ketamine Infusion. Anesthesiology 2018;129(5):1015-1024.

[7] Bosma RL, Kim JA, Cheng JC, Rogachov A, Hemington KS, Osborne NR, Oh J, Davis KD. Dynamic pain connectome functional connectivity and oscillations reflect multiple sclerosis pain. PAIN 2018;159(11):2267-2276.

[8] Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. Nature Reviews Neuroscience 2009;10(3):186-198.

[9] Bullmore ET, Bassett DS. Brain Graphs: Graphical Models of the Human Brain Connectome. Annual Review of Clinical Psychology 2011;7(1):113-140.

[10] Cary RP, Ray S, Grayson DS, Painter J, Carpenter S, Maron L, Sporns O, Stevens AA, Nigg JT, Fair DA. Network Structure among Brain Systems in Adult ADHD is Uniquely Modified by Stimulant Administration. Cereb Cortex 2017;27(8):3970-3979.

[11] Cauda F, D'Agata F, Sacco K, Duca S, Cocito D, Paolasso I, Isoardo G, Geminiani G. Altered resting state attentional networks in diabetic neuropathic pain. 2010;81(7):806-811.

[12] Chai XJ, Castañón AN, Ongür D, Whitfield-Gabrieli S. Anticorrelations in resting state networks without global signal regression. NeuroImage 2012;59(2):1420-1428.

[13] Cheng JC, Bosma RL, Hemington KS, Kucyi A, Lindquist MA, Davis KD. Slow-5 dynamic functional connectivity reflects the capacity to sustain cognitive performance during pain. NeuroImage 2017;157:61-68.

[14] Cheng JC, Rogachov A, Hemington KS, Kucyi A, Bosma RL, Lindquist MA, Inman RD, Davis KD. Multivariate machine learning distinguishes cross-network dynamic functional connectivity patterns in state and trait neuropathic pain. PAIN 2018;159(9):1764-1776.

[15] Combrisson E, Vallat R, O'Reilly C, Jas M, Pascarella A, Saive A-l, Thiery T, Meunier D, Altukhov D, Lajnef T, Ruby P, Guillot A, Jerbi K. Visbrain: A Multi-Purpose GPU-Accelerated Open-Source Suite for Multimodal Brain Data Visualization. 2019;13(14).

[16] Cortes C, Vapnik V. Support-Vector Networks. Machine Learning 1995;20(3):273-297.

[17] Coulombe M-A, Erpelding N, Kucyi A, Davis KD. Intrinsic functional connectivity of periaqueductal gray subregions in humans: PAG Subregional Functional Connectivity. Human Brain Mapping 2016;37(4):1514-1530.

[18] Danon L, Díaz-Guilera A, Duch J, Arenas A. Comparing community structure identification. Journal of Statistical Mechanics: Theory and Experiment 2005;2005(09):P09008-P09008.

[19] Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. Journal of Neuroimmune Pharmacology 2013;8(3):518-534.

[20] Davis KD, Taylor SJ, Crawley AP, Wood ML, Mikulis DJ. Functional MRI of Pain- and Attention-Related Activations in the Human Cingulate Cortex. Journal of Neurophysiology 1997;77(6):3370-3380.

[21] Fillingim RB. Sex, gender, and pain: Women and men really are different. Current Review of Pain, 2000. pp. 4-24.

[22] Fornito A, Zalesky A, Bullmore ET. Chapter 3 - Connectivity Matrices and Brain Graphs. In: A Fornito, A Zalesky, ET Bullmore, editors. Fundamentals of Brain Network Analysis. San Diego: Academic Press, 2016. pp. 89-113.

[23] Fornito A, Zalesky A, Bullmore ET. Chapter 9 - Modularity. Fundamentals of Brain Network Analysis. San Diego: Academic Press, 2016. pp. 303-354.

[24] Fortunato S. Community detection in graphs. Physics Reports 2010;486(3):75-174.

[25] Galli G, Santarnecchi E, Feurra M, Bonifazi M, Rossi S, Paulus MP, Rossi A. Individual and sex-related differences in pain and relief responsiveness are associated with differences in resting-state functional networks in healthy volunteers. European Journal of Neuroscience 2016;43(4):486-493.

[26] Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21(12):2286-2291.

[27] Gómez S, Jensen P, Arenas A. Analysis of community structure in networks of correlated data. Physical Review E 2009;80(1).

[28] Gorgolewski K, Burns CD, Madison C, Clark D, Halchenko YO, Waskom ML, Ghosh SS. Nipype: A Flexible, Lightweight and Extensible Neuroimaging Data Processing Framework in Python. Frontiers in Neuroinformatics 2011;5.

[29] Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, Gold MS, Holdcroft A, Lautenbacher S, Mayer EA, Mogil JS, Murphy AZ, Traub RJ, Consensus Working Group of the Sex G, and Pain SIG of the IASP. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007;132 Suppl 1(Suppl 1):S26-S45.

[30] Guimerà R, Amaral LAN. Cartography of complex networks: modules and universal roles. J Stat Mech 2005;2005(P02001):nihpa35573-nihpa35573.

[31] Guimera R, Sales-Pardo M, Amaral LA. Classes of complex networks defined by role-to-role connectivity profiles. Nature physics 2007;3(1):63.

[32] Gupta A, Kilpatrick L, Labus J, Tillisch K, Braun A, Hong J-Y, Ashe-Mcnalley C, Naliboff B, Mayer EA. Early Adverse Life Events and Resting State Neural Networks in Patients With Chronic Abdominal Pain. 2014;76(6):404-412.

[33] Gupta A, Mayer EA, Fling C, Labus JS, Naliboff BD, Hong J-Y, Kilpatrick LA. Sex-based differences in brain alterations across chronic pain conditions. J Neurosci Res 2017;95(1-2):604-616.

[34] Hashmi JA, Davis KD. Women experience greater heat pain adaptation and habituation than men:. Pain 2009;145(3):350-357.

[35] Hashmi JA, Davis KD. Deconstructing sex differences in pain sensitivity. PAIN 2014;155(1):10-13.

[36] Hashmi JA, Kong J, Spaeth R, Khan S, Kaptchuk TJ, Gollub RL. Functional network architecture predicts psychologically mediated analgesia related to treatment in chronic knee pain patients. Journal of Neuroscience 2014;34(11):3924–3936.

[37] He Y, Lim S, Fortunato S, Sporns O, Zhang L, Qiu J, Xie P, Zuo X-N. Reconfiguration of Cortical Networks in MDD Uncovered by Multiscale Community Detection with fMRI. Cereb Cortex 2018;28(4):1383-1395.

[38] Hemington KS, Rogachov A, Cheng JC, Bosma RL, Kim JA, Osborne NR, Inman RD, Davis KD. Patients with chronic pain exhibit a complex relationship triad between pain, resilience, and within- and cross-network functional connectivity of the default mode network. PAIN 2018;159(8):1621-1630.

[39] Hemington KS, Wu Q, Kucyi A, Inman RD, Davis KD. Abnormal cross-network functional connectivity in chronic pain and its association with clinical symptoms. Brain Structure and Function 2016;221(8):4203-4219.

[40] Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. Moving beyond P values: Everyday data analysis with estimation plots. bioRxiv 2019:377978.

[41] Humphries MD, Gurney K. Network 'Small-World-Ness': A Quantitative Method for Determining Canonical Network Equivalence. PLOS ONE 2008;3(4):e0002051.

[42] Joel D, Berman Z, Tavor I, Wexler N, Gaber O, Stein Y, Shefi N, Pool J, Urchs S, Margulies DS, Liem F, Hänggi J, Jäncke L, Assaf Y. Sex beyond the genitalia: The human brain mosaic. Proceedings of the National Academy of Sciences 2015;112(50):15468-15473.

[43] Kaplan CM, Schrepf A, Vatansever D, Larkin TE, Mawla I, Ichesco E, Kochlefl L, Harte SE, Clauw DJ, Mashour GA, Harris RE. Functional and neurochemical disruptions of brain hub topology in chronic pain. PAIN 2019;160(4):973-983.

[44] Kim JA, Bosma RL, Hemington KS, Rogachov A, Osborne NR, Cheng JC, Oh J, Crawley AP, Dunkley BT, Davis KD. Neuropathic pain and pain interference are linked to alpha-band slowing and reduced beta-band magnetoencephalography activity within the dynamic pain connectome in patients with multiple sclerosis. PAIN 2019;160(1):187-19

[45] Kim JA, Bosma RL, Hemington KS, Rogachov A, Osborne NR, Cheng JC, Oh J, Dunkley BT, Davis KD. Cross-network coupling of neural oscillations in the dynamic pain connectome reflects chronic neuropathic pain in multiple sclerosis. NeuroImage: Clinical 2020;26:102230.

[46] Kucyi A, Davis KD. The dynamic pain connectome. Trends in Neurosciences 2015;38(2):86-95.

[47] Kucyi A, Moayedi M, Weissman-Fogel I, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. Enhanced Medial Prefrontal-Default Mode Network Functional Connectivity

in Chronic Pain and Its Association with Pain Rumination. Journal of Neuroscience 2014;34(11):3969-3975.

[48] Labus JS, Naliboff BN, Fallon J, Berman SM, Suyenobu B, Bueller JA, Mandelkern M, Mayer EA. Sex differences in brain activity during aversive visceral stimulation and its expectation in patients with chronic abdominal pain: a network analysis. NeuroImage 2008;41(3):1032-1043.

[49] Langer N, Pedroni A, Jäncke L. The Problem of Thresholding in Small-World Network Analysis. PLOS ONE 2013;8(1):e53199.

[50] Linden SVD, Valkenburg HA, Cats A. Evaluation of Diagnostic Criteria for Ankylosing Spondylitis. Arthritis & Rheumatism 1984;27(4):361-368.

[51] Liu J, Zhao L, Li G, Xiong S, Nan J, Li J, Yuan K, von Deneen KM, Liang F, Qin W, Tian J. Hierarchical Alteration of Brain Structural and Functional Networks in Female Migraine Sufferers. PLoS ONE 2012;7(12):e51250.

[52] Loggia ML, Kim J, Gollub RL, Vangel MG, Kirsch I, Kong J, Wasan AD, Napadow V. Default mode network connectivity encodes clinical pain: An arterial spin labeling study. Pain 2013;154(1):24-33.

[53] Lotze M, Domin M, Gerlach FH, Gaser C, Lueders E, Schmidt CO, Neumann N. Novel findings from 2,838 Adult Brains on Sex Differences in Gray Matter Brain Volume. Scientific Reports 2019;9(1):1671.

[54] Lutkenhoff ES, Rosenberg M, Chiang J, Zhang K, Pickard JD, Owen AM, Monti MM. Optimized Brain Extraction for Pathological Brains (optiBET). PLOS ONE 2014;9(12):e115551.

[55] Mano H, Kotecha G, Leibnitz K, Matsubara T, Sprenger C, Nakae A, Shenker N, Shibata M, Voon V, Yoshida W, Lee M, Yanagida T, Kawato M, Rosa M, Seymour B. Classification and characterisation of brain network changes in chronic back pain: A multicenter study [version 2; peer review: 3 approved]. Wellcome Open Research 2018;3(19).

[56] Mansour A, Baria AT, Tetreault P, Vachon-Presseau E, Chang P-C, Huang L, Apkarian AV, Baliki MN. Global disruption of degree rank order: a hallmark of chronic pain. Scientific reports 2016;6:34853.

[57] Melzack R. The McGill Pain Questionnaire: Major properties and scoring methods. PAIN 1975;1(3):277-299.

[58] Meunier D, Achard S, Morcom A, Bullmore E. Age-related changes in modular organization of human brain functional networks. NeuroImage 2009;44(3):715-723.

[59] Meunier D, Fonlupt P, Saive A-L, Plailly J, Ravel N, Royet J-P. Modular structure of functional networks in olfactory memory. NeuroImage 2014;95:264-275.

[60] Meunier D, Lambiotte R, Bullmore ET. Modular and Hierarchically Modular Organization of Brain Networks. Frontiers in Neuroscience 2010;4.

[61] Meunier D, Pascarella A, Altukhov D, Jas M, Combrisson E, Lajnef T, Bertrand-Dubois D, Hadid V, Alamian G, Alves J, Barlaam F, Saive A-L, Dehgan A, Jerbi K. NeuroPycon: A free Python toolbox for fast multi-modal and reproducible brain connectivity pipelines. bioRxiv 2019:789842.

[62] Mogil JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nature Reviews Neuroscience 2012;13:859.

[63] Napadow V, Lacount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. Arthritis & Rheumatism 2010;62(8):2545-2555.

[64] Newman MEJ. Assortative Mixing in Networks. Physical Review Letters 2002;89(20):208701.

[65] Newman MEJ. The Structure and Function of Complex Networks. Computer Physics Communications 2003;147:40-45.

[66] Newman MEJ. Modularity and community structure in networks. Proceedings of the National Academy of Sciences 2006;103(23):8577.

[67] Newman MEJ, Girvan M. Finding and evaluating community structure in networks. Physical Review E 2004;69(2):026113.

[68] Osborne NR, Cheng JC, Rogachov A, Kim JA, Hemington KS, Bosma RL, Inman RD, Davis KD. Abnormal subgenual anterior cingulate circuitry is unique to women but not men with chronic pain. PAIN 2020; Online ahead of print.

[69] Peyron R, Faillenot I, Mertens P, Laurent B, Garcia-Larrea L. Motor cortex stimulation in neuropathic pain. Correlations between analgesic effect and hemodynamic changes in the brain. A PET study. NeuroImage 2007;34(1):310-321.

[70] Peyron R, García-Larrea L, Grégoire M-C, Costes N, Convers P, Lavenne F, Mauguière F, Michel D, Laurent B. Haemodynamic brain responses to acute pain in humansSensory and attentional networks. Brain 1999;122(9):1765-1780.

[71] Power Jonathan D, Cohen Alexander L, Nelson Steven M, Wig Gagan S, Barnes Kelly A, Church Jessica A, Vogel Alecia C, Laumann Timothy O, Miezin Fran M, Schlaggar

Bradley L, Petersen Steven E. Functional Network Organization of the Human Brain. Neuron 2011;72(4):665-678.

[72] Power Jonathan D, Schlaggar Bradley L, Lessov-Schlaggar Christina N, Petersen Steven E. Evidence for Hubs in Human Functional Brain Networks. Neuron 2013;79(4):798-813.

[73] Powers D, Ailab. Evaluation: From precision, recall and F-measure to ROC, informedness, markedness & correlation. J Mach Learn Technol 2011;2:2229-3981.

[74] Quesada C, Pommier B, Fauchon C, Bradley C, Créac'h C, Murat M, Vassal F, Peyron R. New procedure of high-frequency repetitive transcranial magnetic stimulation for central neuropathic pain: a placebo-controlled randomized cross-over study. PAIN 2019;Articles in Press.

[75] Raichle ME, Snyder AZ. A default mode of brain function: A brief history of an evolving idea. NeuroImage 2007;37(4):1083-1090.

[76] Rainville P, Duncan GH, Price DD, Carrier Bt, Bushnell MC. Pain Affect Encoded in Human Anterior Cingulate But Not Somatosensory Cortex. Science 1997;277(5328):968.

[77] Ritchie SJ, Cox SR, Shen X, Lombardo MV, Reus LM, Alloza C, Harris MA, Alderson HL, Hunter S, Neilson E, Liewald DCM, Auyeung B, Whalley HC, Lawrie SM, Gale CR, Bastin ME, McIntosh AM, Deary IJ. Sex Differences in the Adult Human Brain: Evidence from 5216 UK Biobank Participants. Cerebral Cortex 2018;28(8):2959-2975.

[78] Rogachov A, Cheng JC, Erpelding N, Hemington KS, Crawley AP, Davis KD. Regional brain signal variability: a novel indicator of pain sensitivity and coping. PAIN 2016;157(11):2483-2492.

[79] Rogachov A, Cheng JC, Hemington KS, Bosma RL, Kim JA, Osborne NR, Inman RD, Davis KD. Abnormal Low-Frequency Oscillations Reflect Trait-Like Pain Ratings in

Chronic Pain Patients Revealed through a Machine Learning Approach. The Journal of Neuroscience 2018;38(33):7293.

[80] Seminowicz DA, Davis KD. Pain Enhances Functional Connectivity of a Brain Network Evoked by Performance of a Cognitive Task. 2007;97(5):3651-3659.

[81] Sha Z, Xia M, Lin Q, Cao M, Tang Y, Xu K, Song H, Wang Z, Wang F, Fox PT, Evans AC, He Y. Meta-Connectomic Analysis Reveals Commonly Disrupted Functional Architectures in Network Modules and Connectors across Brain Disorders. Cerebral Cortex 2017;28(12):4179-4194.

[82] Tagliazucchi E, Balenzuela P, Fraiman D, Chialvo DR. Brain resting state is disrupted in chronic back pain patients. Neuroscience Letters 2010;485(1):26-31.

[83] Tian L, Wang J, Yan C, He Y. Hemisphere- and gender-related differences in small-world brain networks: A resting-state functional MRI study. 2011;54(1):191-202.

[84] Tsubokawa T, Katayama Y, Yamato T, Hirayama T, Koyama S. Treatment of Thalamic Pain by Chronic Motor Cortex Stimulation. Pacing and Clinical Electrophysiology 1991;14(1):131-134.

[85] Vogt BA. Pain and emotion interactions in subregions of the cingulate gyrus. Nature Reviews Neuroscience 2005;6(7):533-544.

[86] Wang G, Erpelding N, Davis KD. Sex differences in connectivity of the subgenual anterior cingulate cortex:. Pain 2014;155(4):755-763.

[87] Watts DJ, Strogatz SH. Collective dynamics of ‘small-world’ networks. Nature 1998;393(6684):440-442.

[88] Weis S, Patil KR, Hoffstaedter F, Nostro A, Yeo BTT, Eickhoff SB. Sex Classification by Resting State Brain Connectivity. Cerebral Cortex 2019.

[89] Yanagisawa T, Fukuma R, Seymour B, Hosomi K, Kishima H, Shimizu T, Yokoi H, Hirata M, Yoshimine T, Kamitani Y, Saitoh Y. Induced sensorimotor brain plasticity controls pain in phantom limb patients. Nature communications 2016;7:13209-13209.

[90] Zhang C, Cahill ND, Arbabshirani MR, White T, Baum SA, Michael AM. Sex and Age Effects of Functional Connectivity in Early Adulthood. 2016;6(9):700-713.

[91] Zhang C, Dougherty CC, Baum SA, White T, Michael AM. Functional connectivity predicts gender: Evidence for gender differences in resting brain connectivity. 2018;39(4):1765-1776.

[92] Zhong J, Chen DQ, Hung PS-P, Hayes DJ, Liang KE, Davis KD, Hodaie M. Multivariate pattern classification of brain white matter connectivity predicts classic trigeminal neuralgia. PAIN 2018;159(10):2076-2087.

Figures Legends

Fig. 1. Modular analysis workflow. (A) fMRI data was acquired during resting state. Nodal parcellation is based on 360 MNI subdivisions of the Human Connectome Project atlas. Edges were defined on the basis of Pearson's correlation coefficients between node BOLD time-series. Link density threshold was applied on each individual correlation matrix on which network analysis and modular detection was performed. Modules are associated with colors and Hubs were defined using two metrics: the within-module degree (WMD) and the participation-coefficient (PC). Provincial Hub (orange circle) has a WMD > 1. Connector Hub (red square) has a WMD > 1 and a large intermodular connectivity with a PC > 0.3. (B) The two graphs show the variations of global modularity and number of modules at 1% to 10% link density (i.e., from 646 to 6462 edges) arising from for males and females healthy controls (grey and blue solid lines) and for those with chronic pain (grey/blue dotted lines) and random network (black). A link density of 5% was used for subsequent analysis.

Fig. 2. The brain network in chronic pain exhibits sex-specific topological organization abnormalities. Data are shown for males (n=45) and females (n=20) with chronic pain (cLBP) and age-sex matched healthy controls (HCs). (A) Normalized mutual information (NMI) in relation to the baseline modular structure (*top-left corner*) of an independent sample of HCs (n=65, control group). Each module is represented by one color. NMI showed a significant chronic pain condition ($F_{(1,126)} = 6.35$; $p = 0.03$) and sex ($F_{(1,126)} = 4.69$; $p = 0.03$) effect with males with cLBP (0.20 ± 0.07), females HCs (0.19 ± 0.09) and females with cLBP (0.16 ± 0.02) exhibiting lower NMI compared to the males HCs (0.23 ± 0.09). In addition, cLBP females

showed significantly lower NMI than cLBP males ($p = 0.016$). No sex*chronic pain interaction ($F_{(2,126)} = 0.11$; $p = 0.74$). The network average within-module connectivity showed a significant interaction between sex and chronic pain factors ($p = 0.03$); whereas the average between-module connectivity was higher in subjects with cLBP compared to males and females HCs. Data shown as mean +/- SD, *significant at $p < 0.05$. (B) The glass brains represent the modular structure in each group at 5% link density, with one color by module. Pie plots showed the number of nodes in each module (among the 360 nodes in the whole-brain). Radar charts showed the proportion of nodes classified as Provincial Hubs (orange filling), peripheral nodes (orange lines), connector Hubs (red filling) and satellite connector (red line), according to the number of nodes in each module.

Fig. 3. Module dissociation index (MDI) in males and females with chronic pain. To quantify the dissociation property for a module, the MDI is calculated as the mean NDI (node dissociation index) for all nodes in one module. The Cohen's d between HCs and patient with cLBP for the 6 modules in common are shown in the above Cumming estimation plot. The raw data is plotted on the upper axes; each mean difference is plotted on the lower axes as a bootstrap sampling distribution. Mean differences between HCs and cLBP in both sexes are depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. Compared to HCs, the MDI in males with cLBP was significantly greater in the SM module, whereas in females with cLBP the MDI was lower in the FP and DM modules.

Fig. 4. Variation between graph metrics and clinical scores. (A) Assortativity and global efficiency were correlated with the disease activity (Bath Ankylosing Spondylitis activity index,

BASDAI) and chronic pain ratings in males with chronic pain but not in females with chronic pain. The other global graph metrics computed did not correlated with these scores. (B) Support Vector Regression between nodal graph metrics (WMD) and clinical score (BASDAI) in males (*Top*, blue) and females (*Bottom*, orange) with chronic pain. Radar charts showed the proportion of brain regions associated with BASDAI for each module (black dotted lines showed the number of nodes). Overall, the WMD of nodes in the: DM, vmPFC and temporal modules in males with cLPB; and in the DM and subcortical modules in females with cLBP, predicted fluctuations of BASDAI scores.

Fig. 5. Summary of the main findings of functional segregations and connectivity changes within networks of the dynamic pain connectome (DPC) [46] in healthy controls (HCs), and those with chronic low back pain (cLBP). Male HCs exhibited distinct modules in the temporal cortex and vmPFC that were associated with a lower engagement of sgACC nodes than in females,; possibly a reflection of their weaker pain habituation (see [35,86]). Female HCs showed distinct fronto-parietal (FP) and subcortical modules, associated with a higher number of connector hubs than found in the males; possibly facilitating engagement of the descending antinociceptive system underlying pain adaptation/habituation(see [35,86]). People with chronic pain of both sexes exhibited global higher cross-network connectivity within the DPC. The sensorimotor module (SM) had higher connectivity with other DPC networks in males with cLBP. Females with cLBP had atypical segregation in the MCC and sgACC that was associated with higher intra-modular connections in the FP and default mode (DM) modules compared to HCs. Brain network modules are associated with one color, dashed lines show higher (red) or lower (grey) connections between males and females HCs or between cLBP and HCs.

Table 1. Common and distinct modules in each group. Four modules have a similar nodes membership in HCs and chronic pain with no sex differences. The temporal and ventro medial prefrontal cortex (vmPFC) modules are specific to males, whereas the frontoparietal and subcortical modules are specific to females. Chronic pain did not change the global modular organization in males, but in females with chronic pain (cLBP) two other modules were detected in the mid cingulate cortex (MCC) and the subgenual anterior cingulate cortex (sgACC).

	Common Modules	**Distinct Modules**	
		Males	Females
HCs	Default Mode (DM) Visual Central Sensorimotor (SM)	Temporal vmPFC	Frontoparietal (FP) Subcortical
cLBP		Temporal vmPFC	Frontoparietal (FP) Subcortical MCC sgACC

Table 2. Support vector machine classification based on nodal graph metrics (i.e., participation coefficient and within-module degree). The within-module degree (WMD) had the best performance to classify participants. Based on univariate feature selection, the top 5% of features were selected and five-fold cross validation was applied (permutation test $p<0.001$). The subjects can be classified according to sex and chronic pain with a good accuracy > 77-92% based on nodal WMD.

SVM Classification	Females vs. males		HCs vs cLBP	
	HC	cLBP	Females	Males
Accuracy	81 %	92 %	85 %	77 %
Nodes	PCC DLPFC ACC TPJ parietal operculum temporal cortex	DLPFC Parietal Subcortical TPJ gyrus frontal inferior	vmPFC S1 DLPFC superior parietal cortex visual cortex lateral temporal cortex	Prefrontal DLPFC OFC ACC frontal operculum