**Supplementary Material**

**Dynamic pain connectome functional connectivity and oscillations reflect multiple sclerosis pain**

Bosma RL1,Kim, JA1,2, Cheng JC1,2, Rogachov A1,2, Hemington KS1,2, Osborne NR1,2, Oh J3, and Davis KD1,2,4

1Division of Brain, Imaging, and Behaviour – Systems Neuroscience, Krembil Research Institute, Toronto Western Hospital, University Health Network, Toronto, Canada

2Institute of Medical Science, University of Toronto, Toronto, Canada

3Keenan Research Centre for Biomedical Science, St. Michael’s Hospital, Toronto, Canada

4Department of Surgery, University of Toronto, Toronto, Canada

**Supplementary Analyses**

To examine the potential confounds of depression, age, disease severity, and head motion we performed several additional analyses:

*Age:*

In terms of age, although the NP group was significantly older than the non-NP group, we do not think that age was driving the differences that we saw between groups because of several analyses that we ran. First, our additional correlation analyses revealed that there was no significant relationship between age and any brain measure (Supplementary Table 1). Second, when we organized the MS data into 2 groups (n = 15, n = 16) by age instead of by NP and non-NP, we did not see any significant brain differences. Third, using age as a covariate, we still found a significant SN-DMN cross-network abnormality in the mixed-NP group (*p* <.001). The cross-network abnormality in the non-NP patients was only a trend (*p* = 0.08) (Figure 2). However, mixed-NP and non-NP groups did not have significantly different SN-DMN sFC (*p* = .7).

Furthermore, we used a second approach which considered age as a covariate in the dFC analyses. For this analysis, the outcome of the results did not change. As seen before, both the mixed-NP and non-NP groups had higher dFC compared to their respective control groups although only the non-NP was significantly different (mixed-NP; *p* = .11, non-NP; *p* = .03). The SN-ascending dFC was not statistically different for the NP versus non-NP subgroups (*p* = .84). Finally, the SN-descending dFC did not differ significantly between patients and control groups (*p* = .24), however, it was significantly attenuated in the NP group compared to the non-NP group (*p* = .04).

*Disease severity:*

Disease severity did not correlate with any brain measure (Supplementary Table 1) and division of groups by disease severity did not demonstrate brain differences (all Ps > .05). However, as we did not have EDSS scores for controls, we were unable to control for this variable as a covariate.

*Depression:*

A closer look at our data showed interesting findings from two types of evaluations of depression. The more widely used measure of depression, the Beck Depression Inventory (BDI), did not indicate any statistically significant differences between groups. The symptoms that the BDI assesses correspond to the DSM-IV criteria for identifying depression, lending to its clinical utility. According to the BDI, both mixed-NP (mean ± SD; 16 ± 9) and non-NP (10 ± 10) groups had average scores between 10-18 which reflect only mild depression. However, the HADS depression scores were slightly different between neuropathic and non-neuropathic groups. The HADS depression scale does not measure the physical symptoms of depression but rather focuses on measurements of emotional distress [[9](#_ENREF_9)]. Therefore, MS patients were only mildly depressed as a group/subgroup and mixed-NP and non-NP subgroups had overlapping depression scores that were not significantly different according to the BDI and only marginally different between HADS scores. Most important was the finding that neither depression score was significantly correlated with any brain measure (p > .05). Including depression as a covariate, the group differences between SN-DMN cross-network connectivity for the mixed-NP group were on the cusp of statistical significance (p= .05). The cross-network abnormality in the non-NP patients was only a trend (p = 0.08) (Figure 2). However, mixed-NP and non-NP groups did not have significantly different SN-DMN sFC (p = .7). Including depression as a covariate in the dFC analyses did not alter the main findings. Both the mixed-NP and non-NP groups had higher SN-ascending dFC compared to their respective control groups although only the non-NP was significantly different (mixed-NP; p = .12, non-NP; p = .03). The SN-ascending dFC was not statistically different for the mixed-NP versus non-NP subgroups (p = .84). Finally, the SN-descending dFC did not differ significantly between patients and control groups (p = .24) however, there was a trend toward an attenuation in the mixed-NP group compared to the non-NP group (p = .08).

*Relationship between FC and painDETECT scores:*

Using a regression analyses we examined the relationship between brain measures and features of neuropathic pain (painDETECT scores) in patients with MS. We included age, depression, disease severity as covariates in the model. Across the whole MS group, there were no significant relationship between functional connectivity or dynamic functional connectivity measures and painDETECT scores, controlling for the covariates (all p > .05).

*Head Motion:*

We implemented several motion correction and physiological noise reduction steps in the preprocessing of the data in order to minimize the potential effects of head motion on the results. Furthermore, independent t-test analyses confirm that there were no significant differences in relative head motion between controls or patients (*p* = .4) or between pain subgroups (*p* = .13). We also included head motion as a covariate in the BOLD variability analyses and correction for mean relative head displacement did not change the significance of the results. Finally, we found that there were no significant relationships between relative head motion and SN-DMN dFC (Rho = -.08, p = .66), SN-Asc dFC (Rho = -.27, p = .13), or SN-Des (Rho = -.21, p = .24).

*Sensory loss and mechanical thresholds*

We examined the mechanical threshold in all patients to assess the issue of sensory loss, known to be an important characteristic of NP [[5](#_ENREF_5)]. The mechanical threshold was determined using calibrated Von Frey filaments on the wrist area of the right hand with the following forces: 0.124, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128, 256, 512 mN. The mechanical threshold was determined by the filament at which the participants indicated that they felt a sensation. We found that patients in both the NP (with scores > 18) and Mixed subgroups (scores 13-18) had significantly higher mechanical detection thresholds compared to the non-NP group (scores < 12) (p = .02, p = .008, respectively). However, there was no difference between the NP and mixed subgroups (p = .90) (see Supplemental Figure 1). This provides additional evidence that the mixed subgroup also has a neuropathic pain component.

*MS pain characteristics*

Common examples of nociceptive pain in our sample were musculoskeletal back pain and migraines, while a common example of neuropathic pain included ongoing extremity pain[[10](#_ENREF_10)]. No clinical assessment of neuropathic pain was conducted, however patients were asked questions regarding their pain history, pain chronicity, and type of pain (Are you currently experiencing pain on a regular basis (“yes” or “no”)? Have you experienced pain on a regular basis in the past 6 months (“yes” or “no”)? Have you ever had pain that lasted more than 3 months (“yes” or “no”)? Describe the types of pain you experience). A summary of their responses are described in Supplementary Table 2.

*Salience network nodes*

To ensure that the rTPJ was working in conjunction with other key nodes of the salience network, as previously described [[1-4](#_ENREF_1); [6](#_ENREF_6); [7](#_ENREF_7)], we conducted functional connectivity analyses between the time series of the rTPJ and the right anterior insula (aINS) and midcingulate cortex (MCC); two other core hubs of this network [[8](#_ENREF_8)]. The time-series of the MCC seed (8 mm ROI located at MNI x = 2, y = 6, x = 36) was highly correlated with the time-series of the rTPJ in both HC (z = .47) and MS patients (z = .46). Similarly, the time-series of the aINS seed (8 mm ROI located at MNI x = 42, y = 10, x = -12) was highly correlated with the time-series of the rTPJ in both HC (z = .56) and MS patients (z = .48). There were no significant differences between the connectivity of the rTPJ-aINS or rTPJ-MCC between MS patients and HC (all p values >.05).

**Supplementary Table 1:** Spearman’s correlation matrix to evaluate the relationship between confounds and brain measures

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | SN\_DMNFC | SN\_ASCFC | SN\_DESFC | SN\_DMNdFC | SN\_ASCdFC | SN\_DesdFC | BOLD VarSw3 | BOLD Varsw4 |
| Age | 0.01 | -0.02 | 0.03 | 0.20 | 0.26 | 0.06 | -0.06 | 0.04 |
| EDSS | 0.1 | .05 | -0.32 | 0.14 | 0.09 | -0.07 | -0.21 | 0.16 |
| Depression  | 0.35 | -0.03 | 0.03 | 0.01 | 0.08 | -0.11 | 0.04 | .27 |

\*Indicates significant correlations

**Supplementary Table 2:** Pain characteristics in MS patients

|  |  |  |
| --- | --- | --- |
|  | Mixed-NP | Non-NP |
| Pain on a regular basis | 80% | 53% |
| Pain on a regular basis > 6m | 87% | 53% |
| Pain lasting > 3m | 67% | 20% |
| Pain in multiple body quadrants | 69% | 40% |
| Average severity (mean ± SD) | 4.2± 2.7 | 2.3± 2.1 |
| Average interference (mean ± SD) | 4.8± 2.8 | 2.0 ± 1.9 |
| Pain detect (mean ± SD) | 19.8 ± 4.1 | 5.3 ± 3.0 |
| Examples | Extremity, neuralgia pain | Back pain, headaches |

**Supplementary Figure 1**:

The mechanical detection thresholds of healthy controls, non-neuropathic pain patients, mixed pain neuropathic patients, and neuropathic pain patients.

**References**

[1] Downar J, Crawley AP, Mikulis DJ, Davis KD. A multimodal cortical network for the detection of changes in the sensory environment. Nat Neurosci 2000;3(3):277-283.

[2] Downar J, Crawley AP, Mikulis DJ, Davis KD. The effect of task relevance on the cortical response to changes in visual and auditory stimuli: an event-related fMRI study. NeuroImage 2001;14(6):1256-1267.

[3] Downar J, Crawley AP, Mikulis DJ, Davis KD. A cortical network sensitive to stimulus salience in a neutral behavioral context across multiple sensory modalities. J Neurophysiol 2002;87(1):615-620.

[4] Downar J, Mikulis DJ, Davis KD. Neural correlates of the prolonged salience of painful stimulation. Neuroimage 2003;20(3):1540-1551.

[5] Finnerup NB, Haroutounian S, Kamerman P, Baron R, Bennett DL, Bouhassira D, Cruccu G, Freeman R, Hansson P, Nurmikko T, Raja SN, Rice AS, Serra J, Smith BH, Treede RD, Jensen TS. Neuropathic pain: an updated grading system for research and clinical practice. Pain 2016;157(8):1599-1606.

[6] Kucyi A, Hodaie M, Davis KD. Lateralization in intrinsic functional connectivity of the temporoparietal junction with salience- and attention-related brain networks. J Neurophysiol 2012;108(12):3382-3392.

[7] Kucyi A, Moayedi M, Weissman-Fogel I, Hodaie M, Davis KD. Hemispheric asymmetry in white matter connectivity of the temporoparietal junction with the insula and prefrontal cortex. PLoS One 2012;7(4):e35589.

[8] Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 2007;27(9):2349-2356.

[9] Smarr KL, Keefer AL. Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). Arthritis Care Res (Hoboken) 2011;63 Suppl 11:S454-466.

[10] Truini A, Barbanti P, Pozzilli C, Cruccu G. A mechanism-based classification of pain in multiple sclerosis. J Neurol 2013;260(2):351-367.