**Supplementary On-line Material**

Differential Effects of Exercise on fMRI of the Midbrain Ascending Arousal Network Nuclei in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) and Gulf War Illness (GWI) in a Model of Postexertional Malaise

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Running title: Midbrain in PEM, ME/CFS and GWI

**BACKGROUND for Figure S1: BOLD of the original region of interest (ROI).**

In a previous study [14] we examined blood oxygenation level dependent (BOLD) activation during a difficult, high cognitive load 2-back task and compared preexercise and postexercise scans. Control, ME/CFS and GWI were equivalent prior to exercise (baseline), but after exercise ME/CFS had a significant increase in BOLD while GWI had a significant decrease in the dorsal midbrain, right middle insula and left Rolandic operculum [14]. The midbrain region of interest extended from the left to right periaqueductal gray (PAG) and to the adjacent right midbrain reticular formation (MRF), inferior colliculus and lateral lemniscus, and caudally to the right lateral isthmus (Figure S1). Because these nuclei have profound influences on threat assessment, pain, negative emotion, attention, wakefulness, and instinctual neurobehaviours, it was of interest to assess the activation of relevant anatomical midbrain nuclei.

**METHOD:**

The original region of interest [14] was portrayed with the ascending arousal network [36,37] on slices in the x, y and z planes.

**RESULTS for Figure S1:**

The seed region approach identified significant differences in BOLD in nuclei that appeared to be outside the region of interest found in the original study (Figure S1). This is possible because > 35 contiguous voxels were required for the ROI analysis and the midbrain activation may have been patchy in the smaller nuclei. It was noted that before exercise ME/CFS had significantly lower BOLD compared to control and GWI when all ascending arousal network nodes were compared. Similarly, after exercise GWI had significantly reduced BOLD compared to ME/CFS. Explanations include exercise-induced changes in cerebral blood flow or neurovascular coupling. Although the same nuclei were impacted, the mechanisms may be unique to each disease.

Figure S1. Midbrain region of interest. The 141 voxel (red) ROI [14] intersected nuclei of the Ascending Arousal Network in the x, y, z planes (MNI coordinate system). In the sagittal slices of the x plane, the ROI extended from the left periaquaductal grey (PAG, white, x = -2.5) through the right midbrain reticular formation (cyan) to the edge of the right pedunculotegmental nucleus (formerly pontopeduncular nucleus, PPN, grey, x=10) and lateral lemniscus at the right lateral margin of the dorsal midbrain (edge at x=12.5). Coronal slices (y plane) showed the right-sided ROI in the region of the inferior colliculus (y = -35), PAG (white), midbrain reticular formation (cyan, y = -29), and cuneiform nucleus (y = -26). Axial slices (z plane) outlined the inferior colliculus (z = -6), PAG (white), midbrain reticular formation (cyan) and region lateral to the dorsal raphe (lime green, z = -15) extending towards the right pedunculotegmental nucleus (grey, z = -15). The ROI was bounded on the rostral side by the superior colliculus, in the ventral midbrain by dorsal raphe (lime green), median raphe (blue), and ventral tegmental area (magenta), and on the caudal end by nuclei in the isthmus of the rostral hindbrain including locus coeruleus (yellow) and adjacent parabrachial complex (dark green) (y = -38). The 141 voxels in the ROI (red) were contiguous. Additional regions with fewer than 35 contiguous voxels that corresponded to other nuclei of the ascending arousal network may have also been activated and significant by the seed region approach but would have not been to small to be significant in the original ROI analysis [14].

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**METHOD for multivariate general linear modeling of demographics and questionnaire data and Tables S1 and S2.**

Preexercise BOLD values were assessed by self-reported demographics variables in multivariable general linear model: Age + gender + PTSD + BMI + Dolorimetry (kg) + Disease status (1 HC, 2 CFS, 3 GWI) + Orthostatic status (1 POTS, 2 START, 3 STOPP) + Marital status (1 single, 2, living together, 3 married, 4 divorced or separated, 5 widow widower) + Home assistance (1 caretaker) + Work status (1 Unemployed, 2 student, 3 Homemaker, 4 retired in good health, 5 Disabled but working, 6 Disabled) + Pain Treatment (0 none,\_1 once per wk, 2 1 or 2 days per week, 3 more than 2 days per week, 4 daily) + Smoking (0 Never, 1 quit 6 months ago, 2\_Yes, still smoking) + Fibromyalgia + Chronic Fatigue Syndrome + Allergic Rhinitis + Sinusitis + Nasal Polyps + Asthma + Depression + Diabetes + Thyroid disease + Bronchitis Emphysema or COPD + Heart disease + High blood pressure + Stroke + Acid reflux ulcers other stomach or intestinal problem + Liver disease + Kidney disease + Back pain Neck + Back pain Middle Back + Back pain Low back + Osteoarthritis degenerative arthritis + Rheumatoid arthritis or other autoimmune disease + Anemia other blood problem or disease + Cancer. The significant covariates were Orthostatic status, low back pain, depression, heart disease, gender and marital status (Table S1).

The next iteration used the significant covariates as fixed factors and removed the other variables. Orthostatic status was the only variable to be significant (Table S2) and was investigated in the other models.

Table S1. Preexercise multivariate general linear modal for BOLD with self-reported demographics as independent variables.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tests of Between-Subjects Effects | Partial Eta Squared | Orthostatic status | Low back pain | CFS | Depression | Heart disease | Gender | Marital status |
| DR | 0.048 |  |  |  |  | 0.026 |  |  |
| L\_MRF | 0.04 |  | 0.042 |  | 0.037 |  |  |  |
| L\_PBC | 0.058 | 0.014 |  |  |  |  |  |  |
| L\_PO | 0.091 |  |  |  |  |  | 0.002 | 0.003 |
| L\_PO | 0.085 |  |  |  |  |  |  |  |
| L\_PPN | 0.058 |  |  |  |  |  |  | 0.014 |
| PAG | 0.058 |  |  |  |  | 0.015 |  |  |
| R\_LC | 0.041 |  |  |  |  |  |  | 0.041 |
| R\_PBC | 0.056 |  |  |  |  |  |  | 0.016 |
| R\_PO | 0.044 |  |  |  |  |  | 0.033 | 0.026 |
| R\_PPN | 0.064 |  |  | 0.01 |  |  |  |  |
| VTA | 0.042 |  |  |  |  |  | 0.038 |  |
|  |  |  |  |  |  |  |  |  |
| Pillai's Trace |  | 0.227 | 0.239 | 0.281 |  | 0.304 | 0.25 | 0.258 |
| F |  | 1.844 | 1.971 | 2.462 |  | 2.748 | 2.093 | 2.182 |
| Hypothesis df |  | 14 | 14 | 14 |  | 14 | 14 | 14 |
| Error df |  | 88 | 88 | 88 |  | 88 | 88 | 88 |
| Sig. |  | 0.044 | 0.029 | 0.006 |  | 0.002 | 0.02 | 0.014 |
| Partial Eta Squared |  | 0.227 | 0.239 | 0.281 |  | 0.304 | 0.25 | 0.258 |

Table S2. Preexercise mGLM of demographic fixed factors. Orthostatic status, Low back pain (LBP), Depression, Heart disease, gender and Marital status were fixed factors with no other covariates.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Multivariate Tests | Effect | Value | F | Hypothesis df | Error df | Sig. | Partial Eta Squared |
| Orthostatic status | Roy's Largest Root | 0.391 | 1.982 | 14 | 71 | 0.032 | 0.281 |
| Gender \* Marital | Roy's Largest Root | 0.414 | 2.100 | 14 | 71 | 0.022 | 0.293 |
| LBP \* Marital | Roy's Largest Root | 0.442 | 2.239 | 14 | 71 | 0.014 | 0.306 |
| LBP \* Gender \* Orthostatic status | Pillai's Trace | 0.498 | 1.681 | 28 | 142 | 0.026 | 0.249 |

**METHOD for Partial correlations and Tables S3 to S6**

Partial correlations compared BOLD signal intensities for each node on Day 1, Day 2 and the delta with subjective questionnaires about CFS symptoms, SF36 domains, psychological and depression complaints with disease status, orthostatic status, gender, age and BMI as covariates.

**RESULTS: for Partial correlations and Tables S3 to S6**

BOLD data were internally correlated within Day 1, Day 2 and delta, positively correlated between Day 2 and delta, and negatively correlated for Day 1 vs delta (Tables S3-S6). There were no significant correlations between subjective questionnaire data and objective preexercise or postexercise BOLD outcomes. The magnitudes of the significant correlations (p < 0.05 corrected) were low (R > 0.4, R < -0.4 for the inversely scored SF36 domains).

SOM Table S3. Partial correlations between BOLD and questionnaire data. Partial correlations were assessed with disease status, orthostatic status, gender, age, PTSD, tenderness by dolorimetry and BMI as covariates. The upper left corner shows correlations for BOLD signal in each node from Day1, Day 2 (cyan edge) and Delta BOLD. BOLD was highly correlated within each day. Delta BOLD was positively correlated with Day 2 and negatively correlated with Day 1. Preexercise was not correlated with postexercise indicating that the exercise protocol induced alterations of the correlation structure. Symptoms were not correlated with BOLD measurements. SF-36 quality of life scores were negatively correlated with the other subjective questionnaires. Nodes and questionnaire items were color coded and are listed below in Tables S2 to S4. Correlations with R > 0.4 (R < -0.4) were significant (p < 0.05 uncorrected).

|  |
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|  |

SOM Table S4. Legend for significance of partial correlations for Table S3.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  | | --- | --- | | Color | R | |  | 1 | |  | > 0.9 | |  | > 0.8 | |  | > 0.7 | |  | > 0.6 | |  | > 0.5 | |  | > 0.4 | |  |  | |  | < -0.4 | |  | < -0.5 | |  | < -0.6 | |  | < -0.7 | |  | < -0.8 | |

SOM Table S5. Partial correlations for Preexercise, Postexercise and Delta BOLD in the upper left corner of Table S3.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Preexercise |  | Postexercise |  | Delta BOLD |  |
| 1 | L\_MRF\_Day1 | 15 | L\_MRF\_Day2 | 29 | L\_MRFΔ |
| 2 | R\_MRF\_Day1 | 16 | R\_MRF\_Day2 | 30 | R\_MRFΔ |
| 3 | VTA\_Day1 | 17 | VTA\_Day2 | 31 | VTAΔ |
| 4 | PAG\_Day1 | 18 | PAG\_Day2 | 32 | PAGΔ |
| 5 | DR\_Day1 | 19 | DR\_Day2 | 33 | DRΔ |
| 6 | MR\_Day1 | 20 | MR\_Day2 | 34 | MRΔ |
| 7 | L\_LC\_Day1 | 21 | L\_LC\_Day2 | 35 | L\_LCΔ |
| 8 | R\_LC\_Day1 | 22 | R\_LC\_Day2 | 36 | R\_LCΔ |
| 9 | L\_PBC\_Day1 | 23 | L\_PBC\_Day2 | 37 | L\_PBCΔ |
| 10 | R\_PBC\_Day1 | 24 | R\_PBC\_Day2 | 38 | R\_PBCΔ |
| 11 | L\_PO\_Day1 | 25 | L\_PO\_Day2 | 39 | L\_POΔ |
| 12 | R\_PO\_Day1 | 26 | R\_PO\_Day2 | 40 | R\_POΔ |
| 13 | L\_PPN\_Day1 | 27 | L\_PPN\_Day2 | 41 | L\_PPNΔ |
| 14 | R\_PPN\_Day1 | 28 | R\_PPN\_Day2 | 42 | R\_PPNΔ |

SOM Table S6. Partial correlations for subjective symptom severities in the lower right corner of Table S3.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  | | --- | --- | | Row Column | Item | | 43 | Migraine | | 44 | Chalder Fatigue Scale | | 45 | CISR | |  |  | | CFS Severity questionnaire | | | 46 | CFSQfatigue | | 47 | CFSQexertion | | 48 | CFSQsleep | | 49 | CFSQmemory | | 50 | CFSQmuscle\_pain | | 51 | CFSQjoint\_pain | | 52 | CFSQsore\_throat | | 53 | CFSQLN | | 54 | CFSQheadaches | |  |  | | SF-36 |  | | 55 | SF36phy\_functioning | | 56 | SF36role\_physical | | 57 | SF36general\_health | | 58 | SF36vitality | | 59 | SF36social\_functioning | | 60 | SF36bodily\_pain | | 61 | SF36role\_emotional | | 62 | SF36mental\_health | |  |  | | 63 | McGill Pain Total | | 64 | General Anxiety GAD7 | |  |  | | Center for Epidemiology Depression | | | 65 | CESD\_∑60 | | 66 | CESD\_Somatic | | 67 | CESD\_Depressed | | 68 | CESD\_Anhedonia | | 69 | CESD\_Interpersonal | | |  |  | | --- | --- | |  |  | | Pain Catastrophizing Score | | | 70 | PCS30:Rumination | | 71 | PCS30:Magnification | | 72 | PCS30:Helplessness | |  |  | | 73 | Irritation Questionnaire | |  |  | | Chronic Multisymptom Inventory | | | 74 | CMSI:Rheum | | 75 | CMSI:SOB | | 76 | CMSI:Cardiac | | 77 | CMSI:HA | | 78 | CMSI:Neuro | | 79 | CMSI:EarSinus | | 80 | CMSI:Rome\_I | | 81 | CMSI:Bladder | | 82 | CMSI:URTI | | 83 | CMSI:Sum | |  |  | | 84 | ΣRhinitis+Chest | | 85 | Irritant Rhinitis Score | |  |  | | Chemical Exposures | | | 86 | C:chemical\_domain | | 87 | C:other\_exposures | | 88 | C:symptoms | | 89 | C:impact\_of\_sensitivities | | 90 | C:masking\_index | |

**METHOD for Preexercise analysis.**

Data were parsed by Disease status and Orthostatic status. Comparisons were made by ANOVA and Tukey Honest Significant Difference and multivariate general linear modeling (SPSS27).

**RESULTS for Preexercise analysis. Tables S7 and S8.**

Orthostatic status preexercise. The only significant difference was POTS greater than STOPP for L\_PBC (mean ± 95%CI, Tukey Honest Significant Difference) (Table S7).

Gender status preexercise found significantly larger. L\_PO in males than females. There was a general trend for greater BOLD in males than females Mean ± 95%CI (Table S8).

SOM Table S7. Orthostatic status preexercise. The only significant difference was POTS greater than STOPP for L\_PBC (mean ± 95%CI, Tukey Honest Significant Difference).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | POTS | START | STOPP | POTS>STOPP |
| N | 21 | 40 | 84 |  |
| L\_MRF | 0.181 ± 0.139 | 0.241 ± 0.105 | 0.174 ± 0.105 |  |
| R\_MRF | 0.201 ± 0.175 | 0.220 ± 0.100 | 0.220 ± 0.100 |  |
| PAG | 0.193 ± 0.188 | 0.239 ± 0.085 | 0.157 ± 0.085 |  |
| VTA | 0.199 ± 0.124 | 0.212 ± 0.068 | 0.175 ± 0.068 |  |
| DR | 0.258 ± 0.162 | 0.268 ± 0.079 | 0.163 ± 0.079 |  |
| MR | 0.328 ± 0.163 | 0.308 ± 0.083 | 0.167 ± 0.083 |  |
| L\_PO | 0.308 ± 0.165 | 0.257 ± 0.083 | 0.162 ± 0.083 |  |
| R\_PO | 0.260 ± 0.147 | 0.314 ± 0.090 | 0.171 ± 0.090 |  |
| L\_PPN | 0.265 ± 0.147 | 0.205 ± 0.084 | 0.219 ± 0.084 |  |
| R\_PPN | 0.328 ± 0.152 | 0.261 ± 0.073 | 0.219 ± 0.073 |  |
| L\_LC | 0.368 ± 0.193 | 0.292 ± 0.103 | 0.154 ± 0.103 |  |
| R\_LC | 0.355 ± 0.196 | 0.393 ± 0.120 | 0.156 ± 0.120 |  |
| L\_PBC | 0.380 ± 0.197 | 0.273 ± 0.091 | 0.146 ± 0.091 | 0.046 |
| R\_PBC | 0.242 ± 0.171 | 0.308 ± 0.099 | 0.185 ± 0.099 |  |

SOM Table S8. Gender status preexercise. L\_PO was significantly larger in males than females. There was a general trend for greater BOLD in males than females. Mean ± 95%CI.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Female | Male | Male > Female |
| N | 54 | 91 |  |
| L\_MRF | 0.158 ± 0.118 | 0.215 ± 0.091 |  |
| R\_MRF | 0.208 ± 0.113 | 0.222 ± 0.096 |  |
| PAG | 0.158 ± 0.104 | 0.201 ± 0.079 |  |
| VTA | 0.149 ± 0.078 | 0.213 ± 0.060 |  |
| DR | 0.156 ± 0.091 | 0.235 ± 0.073 |  |
| MR | 0.177 ± 0.099 | 0.260 ± 0.079 |  |
| L\_PO | 0.116 ± 0.099 | 0.265 ± 0.074 | 0.017 |
| R\_PO | 0.143 ± 0.107 | 0.270 ± 0.080 |  |
| L\_PPN | 0.215 ± 0.095 | 0.226 ± 0.080 |  |
| R\_PPN | 0.220 ± 0.075 | 0.262 ± 0.077 |  |
| L\_LC | 0.139 ± 0.123 | 0.273 ± 0.092 |  |
| R\_LC | 0.186 ± 0.146 | 0.289 ± 0.112 |  |
| L\_PBC | 0.154 ± 0.110 | 0.250 ± 0.085 |  |
| R\_PBC | 0.155 ± 0.123 | 0.270 ± 0.088 |  |

**METHOD for Postexercise analysis. Tables S9 and 10.**

Data were parsed by Disease status and Orthostatic status. Comparisons were made by ANOVA and Tukey Honest Significant Difference and multivariate general linear modeling (SPSS27).

**RESULTS for Postxercise analysis. Tables S9 and 10.**

Orthostatic status postxercise. There were no significant differences based on Orthostatic status following exercise. Mean ± 95%CI. Tukey Honest Significant Difference (Table S9).

Gender status postexercise. There was no differences between women and men following exercise (Table S10)

SOM Table S9. Postexercise orthostatic status.

|  |  |  |  |
| --- | --- | --- | --- |
|  | STOPP | START | POTS |
|  | 84 | 40 | 21 |
| L\_MRF | 0.165 ± 0.090 | 0.200 ± 0.151 | 0.088 ± 0.184 |
| R\_MRF | 0.209 ± 0.089 | 0.204 ± 0.139 | 0.115 ± 0.152 |
| PAG | 0.139 ± 0.081 | 0.190 ± 0.143 | 0.041 ± 0.123 |
| VTA | 0.161 ± 0.067 | 0.147 ± 0.104 | 0.071 ± 0.112 |
| DR | 0.161 ± 0.072 | 0.174 ± 0.123 | 0.068 ± 0.118 |
| MR | 0.182 ± 0.075 | 0.191 ± 0.130 | 0.079 ± 0.140 |
| L\_PO | 0.203 ± 0.082 | 0.125 ± 0.117 | 0.103 ± 0.165 |
| R\_PO | 0.209 ± 0.077 | 0.124 ± 0.124 | 0.110 ± 0.161 |
| L\_PPN | 0.150 ± 0.076 | 0.214 ± 0.129 | 0.038 ± 0.163 |
| R\_PPN | 0.191 ± 0.077 | 0.235 ± 0.125 | 0.206 ± 0.139 |
| L\_LC | 0.204 ± 0.091 | 0.150 ± 0.128 | 0.057 ± 0.177 |
| R\_LC | 0.202 ± 0.099 | 0.144 ± 0.150 | 0.148 ± 0.166 |
| L\_PBC | 0.206 ± 0.084 | 0.172 ± 0.123 | 0.040 ± 0.211 |
| R\_PBC | 0.223 ± 0.079 | 0.122 ± 0.119 | 0.136 ± 0.169 |

SOM Table S10. Postexercise gender status.

|  |  |  |
| --- | --- | --- |
|  | Male | Female |
|  | 91 | 54 |
| L\_MRF | 0.203 ± 0.083 | 0.097 ± 0.127 |
| R\_MRF | 0.219 ± 0.085 | 0.152 ± 0.111 |
| PAG | 0.177 ± 0.075 | 0.074 ± 0.112 |
| VTA | 0.180 ± 0.059 | 0.084 ± 0.090 |
| DR | 0.178 ± 0.067 | 0.107 ± 0.098 |
| MR | 0.192 ± 0.072 | 0.132 ± 0.102 |
| L\_PO | 0.183 ± 0.076 | 0.140 ± 0.105 |
| R\_PO | 0.182 ± 0.072 | 0.153 ± 0.108 |
| L\_PPN | 0.194 ± 0.077 | 0.079 ± 0.097 |
| R\_PPN | 0.248 ± 0.070 | 0.135 ± 0.104 |
| L\_LC | 0.188 ± 0.087 | 0.134 ± 0.109 |
| R\_LC | 0.210 ± 0.088 | 0.124 ± 0.131 |
| L\_PBC | 0.174 ± 0.081 | 0.170 ± 0.114 |
| R\_PBC | 0.210 ± 0.072 | 0.136 ± 0.111 |

**METHODS for Preexercise Multivariate general linear models (mGLM) and Tables S11 to S13.**

Preexercise mGLM used Disease, Orthostatic, PTSD and gender as fixed factors with age, BMI and dolorimetry pressure thresholds as independent variables.

**RESULTS for Preexercise Multivariate general linear models (mGLM) and Table S11 to S13.**

Prior to exercise, Disease status was significant (Wilks’ lambda = 0.662, p = 0.028, Partial Eta Squared = 0.187) (Table S11) with L\_PTN having significantly lower BOLD in CFS (0.018 ± 0.143, mean ± 95%CI) than control (0.326 ± 0.198, p = 0.047 univariate significance) and GWI (0.286 ± 0.127, p = 0.018) (Table S12). This was comparable to the ANOVA outcomes (Table 2).

Orthostatic status was significant (Roy's largest root = 0.256, p = 0.041, Partial Eta Squared = 0.204) with L\_PBC being significantly lower in STOPP (0.062 ± 0.121, mean ± 95%CI) than POTS (0.394 ± 0.219, p = 0.006 Tukey Honest Significant Difference) and START (0.397 ± 0.164, p = 0.034) (Table S13).

Age, gender, PTSD, BMI and dolorimetry pressure thresholds were not significant covariates prior to exercise.

Table S11. Preexercise model. Disease, Orthostatic, PTSD and gender were fixed factors with age, BMI and dolorimetry pressure thresholds as independent variables. Disease status (Wilks' Lambda = 0.662, p = 0.028, Partial Eta Squared = 0.187) and the Orthostatic status \* Gender cross-product (Roy's Largest Root = 0.299, p = 0.014, Partial Eta Squared = 0.23) were significant in the model.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Tests of Between-Subjects Effects | Partial Eta Squared | Disease status | Orthostatic status | PTSD | Orthostatic status \* Gender |
| L\_PPN | 0.109 | 0.001 |  |  |  |
| L\_PBC | 0.071 |  | 0.016 |  |  |
| R\_LC | 0.044 |  |  | 0.024 |  |
| R\_MRF | 0.061 |  |  |  | 0.028 |

Table S12. Disease status in preexercise mGLM. Estimated marginal means (mean ± 95%CI) for Disease status were significantly greater in control and GWI than CFS in L\_PPN (univariate significance). The model contrasts with the differences by ANOVA in Table 2.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Control | CFS | GWI | Control>CFS | GWI>CFS |
| L\_MRF | 0.327 ± 0.248 | 0.152 ± 0.179 | 0.182 ± 0.159 |  |  |
| R\_MRF | 0.461 ± 0.252 | 0.268 ± 0.182 | 0.128 ± 0.162 |  |  |
| PAG | 0.256 ± 0.211 | 0.111 ± 0.152 | 0.232 ± 0.135 |  |  |
| VTA | 0.314 ± 0.164 | 0.104 ± 0.119 | 0.146 ± 0.106 |  |  |
| DR | 0.218 ± 0.191 | 0.120 ± 0.139 | 0.268 ± 0.123 |  |  |
| MR | 0.205 ± 0.206 | 0.144 ± 0.149 | 0.314 ± 0.133 |  |  |
| L\_PO | 0.217 ± 0.193 | 0.056 ± 0.139 | 0.271 ± 0.124 |  |  |
| L\_PPN | 0.326 ± 0.198 | 0.018 ± 0.143 | 0.286 ± 0.127 | 0.047 | 0.018 |
| R\_PO | 0.206 ± 0.214 | 0.116 ± 0.155 | 0.293 ± 0.137 |  |  |
| R\_PPN | 0.384 ± 0.188 | 0.132 ± 0.137 | 0.268 ± 0.121 |  |  |
| L\_LC | 0.160 ± 0.246 | 0.201 ± 0.178 | 0.320 ± 0.158 |  |  |
| R\_LC | 0.047 ± 0.297 | 0.169 ± 0.215 | 0.371 ± 0.191 |  |  |
| L\_PBC | 0.314 ± 0.213 | 0.177 ± 0.155 | 0.317 ± 0.137 |  |  |
| R\_PBC | 0.176 ± 0.242 | 0.130 ± 0.175 | 0.281 ± 0.156 |  |  |

Table S13. Orthostatic status in preexercise mGLM. Estimated marginal means (mean ± 95%CI) for orthostatic status were significantly greater in POTS and START than STOPP in L\_PBC (univariate significance).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | POTS | START | STOPP | POTS>STOPP | START>STOPP |
| L\_MRF | 0.098 ± 0.254 | 0.314 ± 0.190 | 0.210 ± 0.140 |  |  |
| R\_MRF | 0.154 ± 0.258 | 0.360 ± 0.193 | 0.255 ± 0.143 |  |  |
| PAG | 0.104 ± 0.216 | 0.353 ± 0.161 | 0.136 ± 0.120 |  |  |
| VTA | 0.162 ± 0.168 | 0.255 ± 0.126 | 0.125 ± 0.093 |  |  |
| DR | 0.193 ± 0.196 | 0.320 ± 0.146 | 0.117 ± 0.108 |  |  |
| MR | 0.267 ± 0.211 | 0.332 ± 0.158 | 0.110 ± 0.117 |  |  |
| L\_PO | 0.238 ± 0.198 | 0.264 ± 0.148 | 0.090 ± 0.110 |  |  |
| L\_PPN | 0.291 ± 0.202 | 0.242 ± 0.151 | 0.144 ± 0.112 |  |  |
| R\_PO | 0.198 ± 0.219 | 0.326 ± 0.164 | 0.125 ± 0.121 |  |  |
| R\_PPN | 0.288 ± 0.193 | 0.301 ± 0.144 | 0.203 ± 0.106 |  |  |
| L\_LC | 0.358 ± 0.252 | 0.307 ± 0.189 | 0.080 ± 0.139 |  |  |
| R\_LC | 0.255 ± 0.304 | 0.343 ± 0.227 | 0.071 ± 0.168 |  |  |
| L\_PBC | 0.394 ± 0.219 | 0.397 ± 0.164 | 0.062 ± 0.121 | 0.006 | 0.034 |
| R\_PBC | 0.154 ± 0.248 | 0.329 ± 0.186 | 0.131 ± 0.138 |  |  |

**METHODS for Postexercise Multivariate general linear models (mGLM) and Tables S14 to S18.**

Postexercise mGLM used Disease, Orthostatic, PTSD and gender as fixed factors with age, BMI and dolorimetry pressure thresholds as independent variables.

**RESULTS for Postexercise Multivariate general linear models (mGLM) and Table S14 to S18.**

The model was significant for Disease status postexercise (Wilks' lambda = 0.665, p = 0.033, Partial Eta Squared = 0.184). CFS and Control had significantly higher BOLD activation than GWI in L\_MRF, VTA, and R\_PPN (Table S14). CFS was greater than GWI for all except L\_PO, L\_LC and L\_PBC. The mGLM identified more significant nodes than ANOVA (Table 3). 95% confidence intervals for 2-back>0-back condition in GWI bracketed zero suggesting that BOLD may have been equivalent in the 2-back and 0-back trials. In contrast, control and CFS had greater BOLD activation during the high cognitive load 2-back working memory task compared to the low cognitive load 0-back attention task.

The model was significant for gender (Wilks' lambda = 0.798, p = 0.047, Partial Eta Squared = 0.202) (Table S15). Overall, males had greater BOLD activation than females after adjustment for the other variables, but the differences were only significant for R\_LC and R\_PBC.

Postexercise data were reevaluated by regression with BOLD values as the dependent variables, and age, gender, disease status, orthostatic status, PTSD status, BMI, dolorimetry thresholds (kg) as independent variables. The outcome (Table S16) restricted the number of significant regions to six (L\_MRF, R\_MRF, PAG, VTA, DR, R\_PPN ) and three variables.

This restricted model was significant for Disease status (Wilks' lambda = 0, p = 0.007, Partial Eta Squared = 0.981) after accounting for gender and dolorimetry (kg). Estimated marginal means (mean ± 95%CI) were significantly higher in control and CFS than GWI after exercise (univariate significance) (Table S17). The model did not reach significance for gender (Wilks' lambda = 0.161, p = 0.409, Partial Eta Squared = 0.839) but males had a trend for higher BOLD than females in PAG, VTA and R\_PPN by univariate comparisons (Table S18).

The model was significant for dolorimetry thresholds (kg) (Wilks’ lambda = 0, p = 0.049, Partial Eta Squared = 0.984) with significant univariate differences for PAG, VTA, DR and R\_PPN (p < 0.042) (data not shown). However, average dolorimetry results were equivalent in male and female subgroups of control, CFS and GWI by ANOVA in this sample. This results was viewed with skepticism because a larger sample had shown significantly lower pain thresholds in GWI and CFS than control females [25].

Evaluations of postexercise data ended here to avoid overfitting the models.

Table S14. Disease status in postexercise mGLM. The mGLM was significant for Disease status postexercise (Wilks' lambda = 0.665, p = 0.033, Partial Eta Squared = 0.184). CFS and control had significantly higher BOLD activation than GWI.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Control | CFS | GWI | Control>GWI | CFS>GWI |
| L\_MRF | 0.379 ± 0.218 | 0.237 ± 0.157 | -0.045 ± 0.140 | 0.004 | 0.026 |
| R\_MRF | 0.331 ± 0.206 | 0.348 ± 0.149 | 0.042 ± 0.132 |  | 0.008 |
| PAG | 0.204 ± 0.191 | 0.332 ± 0.137 | -0.017 ± 0.122 |  | 0.001 |
| VTA | 0.249 ± 0.160 | 0.202 ± 0.115 | -0.011 ± 0.102 | 0.022 | 0.021 |
| DR | 0.207 ± 0.177 | 0.310 ± 0.129 | -0.016 ± 0.114 |  | 0.001 |
| MR | 0.193 ± 0.194 | 0.322 ± 0.140 | -0.003 ± 0.125 |  | 0.002 |
| L\_PO | 0.109 ± 0.210 | 0.256 ± 0.152 | 0.062 ± 0.135 |  |  |
| L\_PPN | 0.150 ± 0.203 | 0.250 ± 0.146 | -0.022 ± 0.13 |  | 0.02 |
| R\_PO | 0.179 ± 0.199 | 0.276 ± 0.145 | 0.017 ± 0.128 |  | 0.026 |
| R\_PPN | 0.338 ± 0.182 | 0.295 ± 0.132 | 0.067 ± 0.117 | 0.042 | 0.033 |
| L\_LC | 0.124 ± 0.228 | 0.290 ± 0.165 | 0.031 ± 0.147 |  |  |
| R\_LC | 0.133 ± 0.240 | 0.360 ± 0.173 | -0.005 ± 0.154 |  | 0.007 |
| L\_PBC | 0.171 ± 0.220 | 0.267 ± 0.159 | 0.023 ± 0.141 |  |  |
| R\_PBC | 0.147 ± 0.201 | 0.317 ± 0.146 | 0.032 ± 0.13 |  | 0.013 |

Table S15. Gender in postexercise mGLM. The model was significant for gender (Wilks' lambda = 0.798, p = 0.047, Partial Eta Squared = 0.202). Males had higher BOLD activation in R\_LC and R\_PBC than females.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Male | 95%CI | Female |  | Male>Female |
| L\_MRF | 0.206 | 0.127 | 0.114 | 0.144 |  |
| R\_MRF | 0.245 | 0.119 | 0.183 | 0.136 |  |
| PAG | 0.186 | 0.11 | 0.111 | 0.126 |  |
| VTA | 0.186 | 0.093 | 0.071 | 0.106 |  |
| DR | 0.196 | 0.103 | 0.093 | 0.117 |  |
| MR | 0.218 | 0.112 | 0.083 | 0.129 |  |
| L\_PO | 0.194 | 0.123 | 0.077 | 0.14 |  |
| L\_PPN | 0.156 | 0.118 | 0.061 | 0.134 |  |
| R\_PO | 0.191 | 0.116 | 0.091 | 0.133 |  |
| R\_PPN | 0.277 | 0.106 | 0.151 | 0.121 |  |
| L\_LC | 0.226 | 0.133 | 0.049 | 0.151 |  |
| R\_LC | 0.274 | 0.14 | 0.022 | 0.16 | 0.02 |
| L\_PBC | 0.15 | 0.128 | 0.123 | 0.146 |  |
| R\_PBC | 0.255 | 0.117 | 0.052 | 0.134 | 0.026 |

Table S16. Postexercise regression analysis. Postexercise data were reevaluated by regression with BOLD values as the dependent variables, and age, gender, disease status, orthostatic status, PTSD status, BMI, dolorimetry thresholds (kg) as independent variables. The outcome restricted the number of significant nodes and variables to use in the second postexercise multivariate general linear model (Table S17).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Significance for regression model | Univariate significance for Disease status | Univariate significance for dolorimetry | Univariate significance for gender |
| L\_MRF | 0.004 | 0 | 0.031 |  |
| R\_MRF | 0.003 | 0 |  |  |
| PAG | 0.023 | 0.009 | 0.025 |  |
| VTA | 0.002 | 0.001 | 0.017 | 0.014 |
| DR | 0.032 | 0.002 |  |  |
| MR | ns |  |  |  |
| L\_PO | ns |  |  |  |
| R\_PO | ns |  |  |  |
| L\_PPN | ns |  |  |  |
| R\_PPN | 0.005 | 0.001 | 0.028 | 0.016 |
| L\_LC | ns |  |  |  |
| R\_LC | ns |  |  |  |
| L\_PBC | ns |  |  |  |
| R\_PBC | ns |  |  |  |

Table S17. Postexercise multivariate general linear model for Disease status based on regression analysis (Table S16). The model was significant for Disease status (Wilks' lambda = 0, p = 0.007, Partial Eta Squared = 0.981) after accounting for gender and dolorimetry (kg). Estimated marginal means (mean ± 95%CI) were significantly higher in control and CFS than GWI after exercise (univariate significance).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Control | CFS | GWI | Control>GWI | CFS>GWI |
| L\_MRF | 0.341 ± 0.202 | 0.216 ± 0.191 | 0.065 ± 0.130 |  |  |
| R\_MRF | 0.364 ± 0.103 | 0.311 ± 0.098 | 0.066 ± 0.066 | 0.002 | 0.005 |
| PAG | 0.182 ± 0.087 | 0.280 ± 0.083 | 0.055 ± 0.056 |  | 0.003 |
| VTA | 0.252 ± 0.072 | 0.198 ± 0.068 | 0.078 ± 0.047 | 0.006 | 0.032 |
| DR | 0.229 ± 0.079 | 0.269 ± 0.074 | 0.063 ± 0.051 | 0.012 | 0.003 |
| R\_PPN | 0.308 ± 0.080 | 0.291 ± 0.076 | 0.121 ± 0.052 | 0.007 | 0.01 |

Table S18. Postexercise multivariate general linear model for gender based on regression analysis (Table S14). Although the model was not significant for gender (Wilks' lambda = 0.161, p = 0.409, Partial Eta Squared = 0.839), males had greater BOLD than females by univariate significance for PAG, VTA and R\_PPN.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Male | Female | Males>Female |
| L\_MRF | 0.205 ± 0.120 | 0.096 ± 0.154 |  |
| R\_MRF | 0.217 ± 0.062 | 0.154 ± 0.079 |  |
| PAG | 0.178 ± 0.052 | 0.072 ± 0.067 | 0.021 |
| VTA | 0.186 ± 0.042 | 0.080 ± 0.055 | 0.009 |
| DR | 0.178 ± 0.047 | 0.105 ± 0.060 |  |
| R\_PPN | 0.252 ± 0.048 | 0.126 ± 0.061 | 0.006 |

**METHODS for Incremental changes (Postexercise minus Preexercise) by multivariate general linear models (mGLM) and Table S19.**

mGLM for incremental changes in BOLD (ΔBOLD) were evaluated with Disease, Orthostatic, PTSD, gender as fixed factors and age, BMI and dolorimetry as independent variables.

**RESULTS for Incremental changes (Postexercise minus Preexercise) by multivariate general linear models (mGLM) and Table S19.**

The model was significant for Disease status (Wilks' Lambda = 0.664, p = 0.031, Partial Eta Squared = 0.185) and Orthostatic status (Wilks' Lambda = 0.651, p = 0.019, Partial Eta Squared = 0.193) but not age, gender, PTSD, BMI or dolorimetry.

Estimated marginal means for Disease status bracketed zero for controls, were positive for CFS and negative for GWI. Incremental changes were elevated in CFS and decreased in GWI for all nodes except the midbrain reticular formation (Table S19).

Table S19. Estimated marginal means for ΔBOLD and Disease status. Mean ± 95%CI. Univariate significance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Control | CFS | GWI | CFS > GWI |
| L\_MRFΔ | 0.052 ± 0.307 | 0.085 ± 0.222 | -0.227 ± 0.198 |  |
| R\_MRFΔ | -0.131 ± 0.312 | 0.080 ± 0.226 | -0.086 ± 0.201 |  |
| PAGΔ | -0.052 ± 0.264 | 0.221 ± 0.191 | -0.249 ± 0.170 | 0.001 |
| VTAΔ | -0.065 ± 0.206 | 0.098 ± 0.148 | -0.157 ± 0.132 | 0.035 |
| DRΔ | -0.012 ± 0.245 | 0.190 ± 0.178 | -0.283 ± 0.158 | 0.00036 |
| MRΔ | -0.012 ± 0.264 | 0.178 ± 0.192 | -0.317 ± 0.170 | 0.001 |
| L\_POΔ | -0.108 ± 0.267 | 0.200 ± 0.194 | -0.209 ± 0.171 | 0.006 |
| L\_PPNΔ | -0.176 ± 0.250 | 0.232 ± 0.181 | -0.308 ± 0.160 | 0.000059 |
| R\_POΔ | -0.027 ± 0.290 | 0.160 ± 0.211 | -0.276 ± 0.187 | 0.007 |
| R\_PPNΔ | -0.046 ± 0.254 | 0.163 ± 0.184 | -0.201 ± 0.164 | 0.011 |
| L\_LCΔ | -0.037 ± 0.306 | 0.089 ± 0.222 | -0.290 ± 0.197 | 0.036 |
| R\_LCΔ | 0.086 ± 0.381 | 0.192 ± 0.276 | -0.376 ± 0.245 | 0.008 |
| L\_PBCΔ | -0.143 ± 0.295 | 0.090 ± 0.213 | -0.294 ± 0.189 | 0.025 |
| R\_PBCΔ | -0.029 ± 0.308 | 0.187 ± 0.223 | -0.249 ± 0.198 | 0.013 |