**Supplemental Material**

**Materials and Methods**

Subjects underwent ECG recordings at baseline prior to start of therapy. All ECG recordings were performed under controlled experimental settings in the pediatric Translational Research Unit at Children’s Hospital of Wisconsin. Subjects were asked to refrain from caffeine intake on the day of the recordings. No changes to pharmacologic therapy were permitted for two consecutive weeks prior to the start of study procedures. After a period of 10 min supine rest in a quiet room, ECG recording was performed in supine, sitting, and standing cued posture shifts for 3 min in each position. Raw ECG data was acquired with an Actiwave single channel ECG recorder (CamNtec Inc, Boerne, TX, USA) at a sampling rate of 1000Hz. Inter-beat intervals were identified as the timing between R-waves. Prior to analysis, data were manually inspected for artifacts, arrhythmias, and missed beats, which can lead to bias and invalidate beat-to-beat measures of heart rate and related measures.1 Missed beats were reconstructed, noise artifacts removed, and IBI fluctuations due to ventricular arrhythmias were removed to produce a clean, uninterrupted beat-to-beat signal using CardioEdit+ Software (Brain-Body Center for Psychophysiology and Bioengineering, Department of Psychiatry, University of North Carolina at Chapel Hill). The following measures were computed:

*Heart period (HP).* HP is the duration of time between successive heart beats, measured in milliseconds. HP is the reciprocal of heart rate and was selected based on evidence that HP has a stronger linear relation with autonomic control than heart rate and better distributional features for parametric analysis.2 HP data were time sampled at 4 Hz for all analyses. The mean of 15-sec epochs was calculated for each 3-minute posture, excluding the transitions between them.

*Respiratory sinus arrhythmia (RSA) amplitude.* RSA is a measure of cardiac vagal tone, the effect of the myelinated vagus nerve on the heart, measured in units of ln(ms2).3,4 Calculations were performed using the Porges-Bohrer method,

5,6 which employs a time-frequency method to extract a frequency band-limited component from the heart period time series that represents RSA. This validated method has high sensitivity for quantifying cardiac vagal influences.5,7 Its quantitative advantage permits RSA quantification even when the signal is weak or superimposed over an aperiodic non-stationary baseline trend. The method used a 51-point (i.e., 12.75 second duration) moving polynomial filter to remove variance associated with complex aperiodic shifts and oscillations slower than the respiratory frequency. The residual output of this process was band-passed and the heart period variance in the frequency band associated with spontaneous breathing in adolescents (.12–1.02 Hz) was extracted. The variance of this result is natural log transformed to reduce skewness in the RSA metric. The mean of 15-sec epochs was calculated for each 3-minute posture, excluding the transitions between them.

*Vagal efficiency (VE).* A measure of the influence of cardiac vagal tone on heart rate was calculated from all sequential 15-sec epoch estimates of HP and RSA within each of the three posture segments. The slope from regression analyses between the total set of HP and RSA values within each file defines vagal efficiency, an index of the involvement of the myelinated vagus in the dynamic regulation of the heart (Figure 1).8

*Pain assessment.* Patients completed the self-reported Pain Frequency-Severity-Duration (PFSD) scale at baseline, weekly during therapy and at the follow-up visit as previously described.9

Statistical analysis:

Analysis was conducted in R 3.6.1 and RStudio 1.2.10 The lme4 package11 was used for mixed effects modeling to examine the interaction of the baseline assessment vagal efficiency and treatment group on pain ratings (supplemental material). Parametric bootstrapping with 10,000 samples was used to compute 95% confidence intervals.12

Treatment response was analyzed using mixed effects models. The base structure for models was:

where i is the observation, j is the individual, pain is the PFSD composite, time is the time point (pre-therapy baseline, after 3 weeks of therapy, and post-therapy – designated by values of m), Baseline Physio is the physiological indicator at baseline (Respiratory sinus arrhythmia, heart period, or vagal efficiency), u is the random individual-level intercept, and r is the residual.

**Results**

Two extreme outliers on the VE measure were removed from the analysis (VE = -.33.45 [2.76 SD below the mean] and VE = 162.25 [3 SD above the mean]), both from the treatment group.



**Consort Diagram**. Consort flow diagram outlining subject allocation, dropouts and exclusions from original study population.9

**Table X.** Physiological predictors at baseline.

| **Variable** | **n** | **Mean** | **SD** | **Median** | **Min** | **Max** |
| --- | --- | --- | --- | --- | --- | --- |
| Heart period (HP; ms) |  |  |  |  |  |  |
| Supine | 92 | 797.98 | 128.08 | 782.72 | 537.01 | 1153.04 |
| Seated | 92 | 711.38 | 111.9 | 685.9 | 505.14 | 992.73 |
| Standing | 92 | 625.68 | 99.44 | 603.77 | 426.8 | 870.03 |
| Respiratory Sinus Arrhythmia (RSA; ln(ms)2) |  |  |  |  |  |  |
| Supine | 92 | 6.41 | 1.29 | 6.55 | 3.08 | 9.45 |
| Seated | 92 | 5.85 | 1.08 | 5.81 | 3.58 | 8.77 |
| Standing | 91 | 4.98 | 1.52 | 4.99 | 2.00 | 10.24 |
| Vagal Efficiency (HP-RSA slope) | 90 | 54.04 | 28.58 | 51.83 | -5.48 | 132.43 |

**Table Y.** Physiological predictors by treatment group

|  | **PENFS** | | **Sham** | | |  | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Mean** | **SD** | | **Mean** | **SD** | | **Difference Test** |
| Heart period (HP; ms) |  |  | |  |  | |  |
| Supine | 814.90 | 141.96 | | 780.31 | 110.61 | | t(90) = -1.30, p = .20 |
| Seated | 714.03 | 117.08 | | 708.62 | 107.48 | | t(90) = -.23, p = .82 |
| Standing | 628.92 | 99.80 | | 622.30 | 100.08 | | t(90) = -.32, p = .75 |
| Respiratory Sinus Arrhythmia (RSA; ln(ms)2) |  |  | |  |  | |  |
| Supine | 6.49 | 1.25 | | 6.32 | 1.34 | | t(90) = -.63, p = .53 |
| Seated | 5.85 | 1.09 | | 5.85 | 1.09 | | t(90) = -.01, p = .99 |
| Standing | 5.03 | 1.59 | | 4.92 | 1.46 | | t(89) = -.34, p = .74 |
| Vagal Efficiency (HP-RSA slope) | 57.22 | 29.29 | | 50.87 | 27.81 | | t(88) = -1.05, p = .29 |

**Table Z.** Baseline physiological variable correlations.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **1** | **2** | **3** | **4** | **5** | **6** |
| *Heart Period (HP)* |  |  |  |  |  |  |
| 1. Supine |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| 2. Seated | .85\*\* |  |  |  |  |  |
|  | [.78, .90] |  |  |  |  |  |
|  |  |  |  |  |  |  |
| 3. Standing | .61\*\* | .73\*\* |  |  |  |  |
|  | [.46, .72] | [.61, .81] |  |  |  |  |
|  |  |  |  |  |  |  |
| *Respiratory Sinus Arrhythmia (RSA)* |  |  |  |  |  |  |
| 4. Supine | .61\*\* | .45\*\* | .35\*\* |  |  |  |
|  | [.46, .72] | [.27, .60] | [.16, .52] |  |  |  |
|  |  |  |  |  |  |  |
| 5. Seated | .58\*\* | .71\*\* | .62\*\* | .65\*\* |  |  |
|  | [.42, .70] | [.59, .80] | [.48, .73] | [.52, .76] |  |  |
|  |  |  |  |  |  |  |
| 6. Standing | .32\*\* | .40\*\* | .75\*\* | .42\*\* | .68\*\* |  |
|  | [.13, .50] | [.21, .56] | [.64, .83] | [.23, .57] | [.55, .78] |  |
|  |  |  |  |  |  |  |
| *Vagal Efficiency (VE)* |  |  |  |  |  |  |
| 7. HP-RSA Slope | .62\*\* | .41\*\* | .14 | .57\*\* | .34\*\* | .08 |
|  | [.47, .73] | [.22, .56] | [-.06, .34] | [.42, .70] | [.15, .51] | [-.13, .28] |
|  |  |  |  |  |  |  |

*Note.* Values in square brackets describe the 95% confidence interval for each correlation. \* *p* < .05.

\*\* *p* < .01.



**Figure X.** Percutaneous electrical nerve

field stimulation (PENFS) device.

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**Figure Y.** Plot of vagal efficiency and residualized pain composite change scores by treatment group. Pain change scores from baseline to week 3 were regressed on baseline scores to remove effect of baseline scores, and residualized values saved. These residualized pain change values were compared with baseline vagal efficiency scores. There was a positive correlation between vagal efficiency and residualized pain change scores in the treatment group (r = .39, p = .011) but not the placebo group (r = .01, p = .957). Positive pain change values reflect increased pain, 0 reflect no change (marked by a dotted line), and negative scores reflect pain reduction.

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