Supplementary Online Content


**eMethods.** Overall Study Methods

**eTable.** Parameter Estimates for Exploratory Multivariate Logistic Regressions Including the LF:HF Ratio and Each Covariate of Interest in Predicting No PTSD Diagnosis Before Deployment to PTSD After Deployment in MRS-I and MRS-II Participants

This supplementary material has been provided by the authors to give readers additional information about their work.
**eMethods.** Overall Study Methods

**HRV Frequency-Domain Measures Definitions**

1) LF: absolute power of the low frequency (0.04-0.15 Hz) band in ms²; a frequency-domain measure thought to reflect sympathetic activity and some parasympathetic activity \(^1,^2\);

2) HF: absolute power of the high frequency (0.15-0.4 Hz) band in ms²; a frequency-domain measure thought to reflect primarily parasympathetic activity \(^1,^2\);

3) LF:HF ratio: ratio of LF over HF; a frequency-domain measure. Higher ratios have been proposed to reflect more sympathetic relative to parasympathetic activity \(^3\) for review of interpretation of LF:HF ratio see \(^4\).

**PPG and Study Procedure**

Fluctuations in the blood volume of the finger are directly related to the activity of the heart, thus the interval between peaks in the PPG signal, known as the PP interval, is considered a reasonably accurate reflection of the RR interval \(^5\). PPG has been shown to be a sensitive and reliable peripheral instrument for the capture of cardiac activity \(^6\), for example it is highly correlated with waveforms from simultaneous ECG recordings \(^7,^8\). Frequency and time-domain measures of HRV derived from PPG were not significantly different from those derived by a simultaneous two-lead ECG recording \(^5\). PPG was sampled at the rate of 1000 Hz. Using an oscilloscope display and amplification of the PPG signal (San Diego Instruments), the examiner ensured that the PPG was adequately capturing the heart beat waveforms without cutting off the peak of the R wave. The position of the PPG was adjusted until a visually clear heart-beat signal was obtained and each participant was asked to keep his hand relatively motionless during the 5 min recording.

Participants were asked to sit comfortably and direct their attention to a computer monitor where they were entertained with simple visual puzzles (e.g., locating hidden images in...
a photograph). The images were selected to be neutral and minimally affectively arousing or stress-inducing (e.g., dolphins, frogs). They were told that they did not need to memorize anything and that they would not be tested on the images afterward. The purpose of the hidden image task was to maximize the likelihood that participants remained stationary, awake, and alert for the duration of the recording. The images changed every 60 seconds (thus outside of the bandwidth for both LF and HF frequency ranges—see above) and the order of presentation of the images was the same for all participants. To minimize uncontrolled acoustic stimuli in the testing environment subjects were fitted with headphones producing a 70 dB broad band background noise (see9 for details).

Deployment Risk and Resilience Inventory (DRRI)

Four DRRI subscales were used to measure deployment stressors: Combat Experiences, Aftermath of Battle, Deployment Concerns about Life and Family Disruptions and the Difficulty Living and Working Environments. Because each scale varies somewhat in score range, scores for each DRRI subscale were converted to z-scores (subject raw score – group mean score) and then averaged to produce one composite DRRI score with equal weighting across scales 10. Positive and negative values represent higher and lower deployment stress, respectively (-2, 3 = Min, Max). This composite approach was chosen because multiple deployment factors predict PTSD in addition to combat experience 11, and the composite score had the highest correlation with post-deployment PTSD symptoms compared to any single scale in our sample.

HRV Data Processing

1) The systolic peaks of the PPG signal were identified and a tachogram representing intervals between heartbeats (the PP interval) was generated by measuring the time difference between successive peaks.
2) Tachograms were processed by the HRV analysis module of VivoSense 1.0 (Vivonoetics, 2011), which can process multiple files in an automated “batch process” format. For each file in the batch process the software automatically performed the following steps: (a) Peak detection of the PPG waveform; (b) Automatic detection and labeling of potential motion artifact. This procedure was done by setting an upper and lower threshold on the PP interval for each beat. If the PP interval exceeded physiological limits the beat was flagged as potential artifact. The limits were set as the median PP interval +/- 0.5 s; (c) Automatic editing of artifact. If an artifact was less than 3 s, the PP time series was interpolated with a linear interpolation routine. If the artifact was longer than 3 s the region was excluded from the analysis and annotated to note that it was excluded. (d) Instantaneous heart rate determination in beats per minute as 60/PP. Average heart rate over the 5-minute interval was then reported by taking the arithmetic mean of the instantaneous heart rate; (e) Frequency domain HRV indices were computed on the PP tachogram for the entire 5-minute time interval. Derivation of HRV followed the procedures described in the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommendations for short-term HRV recordings. Frequency-domain measures were derived from a Welch Periodogram and included the following steps: The PP time series was first resampled to an even sample rate of 4s. Resampling was performed using a linear interpolation routine. The PP time series was divided into overlapping windows of 256 samples in length with 75% overlap. Each window was multiplied by a Hanning window to reduce spectral leakage. A linear trend for each window was subtracted to eliminate the DC spectral component. The fast Fourier Transform (FFT) of each of windowing was calculated and the squared magnitude obtained. Each window was scaled and averaged window to result in the Welch periodogram. Any windows containing regions that were flagged for exclusion were eliminated from the average to ensure that results were still available in the presence of a discontinuity. The periodogram was integrated between defined frequency ranges to obtain the final HRV indices. For example these ranges were the
recommended 0.04-0.15 Hz for the low frequency index (LF) and 0.15-0.4 Hz for the high frequency index (HF).

3) Following the batch process, trained scorers (VR, AM) visually inspected each file in accordance with the recommendation that automated HRV analyses should be followed by visual inspection and manual correction. Additional corrections were required in approximately 8%-10% of files and each corrected file was re-processed to generate HRV variables.

4) Files for which the software determined that there was insufficient artifact-free data to accurately calculate frequency-domain variables were excluded from further HRV analysis. Typically this occurred when there was prominent motion artifact throughout the 5-minute session. The HRV data for 5.4% of MRS-I participants were not included.

**Genetic-Based Ancestry**

Using blood samples, genotypes of 1,783 ancestry-informative markers (AIMs) were used to determine a participant's ancestry at the continental level. Ancestry estimates were determined using STRUCTURE v2.3.2.1.\textsuperscript{13} at K=7, including prior population information of the HGDP reference set \textsuperscript{14}. Based on these ancestry estimates, participants were placed into 4 groups: participants with >95% European ancestry were grouped with European-Americans; participants with >5% African ancestry and <5% each Native American, Central Asian, East Asian, and Oceanic ancestry as African-American, participants with >5% Native American and <10% African, and <5% each Central Asian, East Asian and Oceanic ancestry as Hispanic and Native Americans, and all others.

**Regression Model Shrinkage and Performance**

To assess if there was overfitting of the regression model, the model was refit using penalized maximum likelihood estimation with the glmnet R package \textsuperscript{15}. Parameter shrinkage was evaluated under both lasso and ridge penalties. Lasso and ridge penalty fitted estimates of
the log-transformed LF:HF ratio effect size were respectively 99% and 94% of the effect size estimated under the original logistic model.

To assess the performance of the regression model, a receiver-operating-characteristic (ROC) analysis of the regression models was performed using the pROC R package. PTSD status and fitted probabilities from the regression model were used to derive the ROC curve. Using just the log-transformed LF:HF ratio in the model and pooling the two ROC curves from MRS-I and MRS-II, the AUC was 55.73 (95% CI = 49.33-62.14), indicating that LF:HF is a modest contributor to the overall prediction of PTSD risk. Using all covariates, the area under the curve (AUC) was 81.71 (95% CI = 75.24 - 88.18).

Post-Hoc Analyses

Post-hoc correlations were conducted between pre-deployment HRV variables and the following variables: total scores on the Alcohol Use Identification Disorders Test (AUDIT), hours since nicotine use for those participants who reported using nicotine, and hours since caffeine use for those participants who reported using caffeine. Consistent with our previous findings, statistically significant positive correlations were observed between log-transformed LF and hours since nicotine use (Pearson r = 0.11, p = .004) and between log-transformed HF and hours since nicotine use (Pearson r =0.09, P = .013). The correlations between HRV measures and AUDIT scores or caffeine use did not reach or approach statistical significance. Post-hoc correlations were also conducted between pre-deployment HRV and post-deployment DRRI scores. The only HRV variable to reach statistical significance, albeit with a small effect size, was log-transformed LF; lower LF at pre-deployment was associated with higher DRRI scores at post-deployment (Pearson r = -0.08, P = .006).

As expected, the 3 HRV variables were significantly intercorrelated, e.g., there was a positive correlation between the log-transformed LF:HF ratio and log-transformed LF.
r=0.22, P < .001), a negative correlation between LF:HF and HF (Pearson r=-0.56, P < .001), and a positive correlation between LF and HF (Pearson r=0.65, P < .001).

To generate hypotheses for future studies, we sought to determine the extent to which covariates previously associated with HRV (age) and PTSD (pre-deployment CAPS scores, deployment-related DRRI scores, deployment-related TBI) may have each influenced the relationship between the pre-deployment LF:HF ratio and post-deployment PTSD. A series of post-hoc exploratory logistic regressions were conducted where post-deployment PTSD was the outcome and the predictors in each regression included the log-transformed LF:HF ratio (centered around its mean using the scale function in the statistical program R), the covariate of interest, and the interaction of the LF:HF ratio with the covariate of interest. The MRS-I and MRS-II data were pooled for these analyses. The eTable presents the results. The LF:HF ratio remained significant in all models except in the pre-deployment CAPS score model where the LF:HF ratio approached significance (P = .07). Thus pre-deployment CAPS scores may in part moderate the effect of HRV on post-deployment PTSD. No interactions between the LF:HF ratio and covariates reached significance.

To determine whether recency of nicotine or caffeine use may have impacted the findings, the logistic regression on the log-transformed LF:HF ratio and all covariates was repeated with the addition of categorical variables for recency of nicotine use and recency of caffeine use (categories from 1 to 5, 1=use within two hours, 5=no use for 12 hours or more). The LF:HF ratio achieved statistical significance in this model (odds ratio=1.65, P = .02).

To determine whether the exclusion of HRV outliers influenced the results, the MRS-I regression was repeated with the inclusion of three participants who had outlier values for the LF:HF ratio, and the significance value of the LF:HF ratio did not change (odds ratio=1.63, P = .008). Finally, as a supplement to the MRS-I and MRS-II meta-analysis, the MRS-I and MRS-II cohorts were combined and the logistic regression was repeated. DRRI composite scores and the log-transformed LF:HF ratio were included in the model. The odds ratio and significance
value for LF:HF was similar to those of the meta-analysis (odds ratio=1.45, \( P = .012 \)). This combined analysis was conducted again with the inclusion of participants (3 in MRS-I, 3 in MRS-II) who had outlier values for LF:HF; again the LF:HF ratio retained significance in the model (odds ratio=1.46, \( P = .011 \)).
**eTable.** Parameter estimates for exploratory multivariate logistic regressions including the LF:HF ratio and each covariate of interest in predicting no PTSD diagnosis before deployment to PTSD after deployment in MRS-I and MRS-II participants.

<table>
<thead>
<tr>
<th>Covariate precedes HRV</th>
<th>Age</th>
<th>Exp (B) Odds Ratio</th>
<th>Wald $\chi^2$</th>
<th>Sig.</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.82</td>
<td>1.84</td>
<td>0.18</td>
<td>0.59</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>LF:HF ratio</td>
<td>1.37</td>
<td>7.54</td>
<td>0.006</td>
<td>1.10</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>Age x LF:HF ratio</td>
<td>1.08</td>
<td>0.45</td>
<td>0.50</td>
<td>0.85</td>
<td>1.36</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Covariate coincident with HRV</th>
<th>Pre-deployment CAPS score</th>
<th>Exp (B) Odds Ratio</th>
<th>Wald $\chi^2$</th>
<th>Sig.</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPS score</td>
<td>1.66</td>
<td>30.91</td>
<td>&lt;0 .001</td>
<td>1.38</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>LF:HF ratio</td>
<td>1.26</td>
<td>3.38</td>
<td>0.07</td>
<td>0.99</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>CAPS score x LF:HF ratio</td>
<td>0.99</td>
<td>0.002</td>
<td>0.96</td>
<td>0.84</td>
<td>1.18</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Covariates intermediate between HRV and PTSD</th>
<th>Deployment-Related TBI</th>
<th>Exp (B) Odds Ratio</th>
<th>Wald $\chi^2$</th>
<th>Sig.</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBI</td>
<td>5.77</td>
<td>46.38</td>
<td>&lt; 0.001</td>
<td>3.50</td>
<td>9.65</td>
<td></td>
</tr>
<tr>
<td>LF:HF ratio</td>
<td>1.59</td>
<td>6.64</td>
<td>0.01</td>
<td>1.12</td>
<td>2.28</td>
<td></td>
</tr>
<tr>
<td>TBI x LF:HF ratio</td>
<td>0.71</td>
<td>1.76</td>
<td>0.19</td>
<td>0.43</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exp (B) Odds Ratio</td>
<td>Wald $\chi^2$</td>
<td>Sig.</td>
<td>Lower CI</td>
<td>Upper CI</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------</td>
<td>---------------</td>
<td>------</td>
<td>----------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>DRRI score</td>
<td>2.32</td>
<td>54.80</td>
<td>&lt; 0.001</td>
<td>1.86</td>
<td>2.91</td>
<td></td>
</tr>
<tr>
<td>LF:HF ratio</td>
<td>1.53</td>
<td>8.34</td>
<td>0.004</td>
<td>1.15</td>
<td>2.04</td>
<td></td>
</tr>
<tr>
<td>DRRI score x LF:HF ratio</td>
<td>0.84</td>
<td>2.26</td>
<td>0.13</td>
<td>0.68</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

CAPS = Clinician-Administered PTSD Scale, DRRI = Deployment Risk and Resilience Inventory, CI = 95% confidence interval. The LF:HF ratio was log-transformed and centered around its mean. Ordinal variables (age, pre-deployment CAPS scores, DRRI scores) were coded as deviations from their mean.
eReferences


