Inclusion of Respiratory Frequency Information in Heart Rate Variability Analysis for Stress Assessment

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***Abstract*—Respiratory rate and heart rate variability (HRV) are studied as stress markers in a database of young healthy volunteers subjected to acute emotional stress, induced by a modification of the Trier Social Stress Test. First, instantaneous frequency domain HRV parameters are computed using time-frequency analysis in the classical bands. Then, the respiratory rate is estimated and this information is included in HRV analysis in two ways: 1) redefining the high-frequency (HF) band to be centered at respiratory frequency; 2) excluding from the analysis those instants where respiratory frequency falls within the low-frequency (LF) band. Classical frequency domain HRV indices scarcely show statistical differences during stress. However, when including respiratory frequency information in HRV analysis, the normalized LF power as well as the LF/HF ratio significantly increase during stress (p-value < 0.05 according to the Wilcoxon test), revealing higher sympathetic dominance. The LF power increases during stress, only being significantly different in a stress anticipation stage, while the HF power decreases during stress, only being significantly different during the stress task demanding attention. Our results support that joint analysis of respiration and HRV obtains a more reliable characterization of autonomic nervous response to stress. In addition, the respiratory rate is observed to be higher and less stable during stress than during relax (p-value < 0.05 according to the Wilcoxon test) being the most discriminative index for stress stratification (AUC $= 88.2\%$).**

***Index Terms*—Autonomic Nervous System, heart rate variability, respiration, stress, sympathovagal balance, time-frequency methods.**

I. Introduction

STRESS is the physiological response to a threat, either physical or psychological, mainly mediated by the autonomic nervous system (ANS) through its two branches, sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). This response starts in the hypothalamus, which triggers the sympathetic "fight or flight" response to provide the body with the energy to address the perceived danger. Once the threat has passed, the parasympathetic "rest and digest" response restores the body homeostasis. In this way, stress is a necessary survival mechanism and not health-threatening.

However, when stress response is maintained in time or it is initiated over and over again, the body cannot reach its homeostasis. Prolonged stress has been associated with dysfunctions in the immune system [1], psychiatric disorders such as anxiety, depression, and Alzheimer [2], [3], and cardiovascular diseases [4], [5]. The World Health Organization has called stress the health epidemic of the 21st century. The identification of human daily stress level would be very useful for continuous management of the stress.

Despite the high incidence and negative consequences of stress, there is not a reliable tool for the noninvasive, objective, and continuous monitoring of stress level. The goal of ES3 project is creating such a tool, which includes different physiological signals, biochemical markers, and psychometric questionnaires, during physical, emotional, and even chronic stress [6]. In this paper, we will focus on acute emotional stress.

Heart rate variability (HRV) at rest is widely accepted as a noninvasive measure of the ANS regulation of the heart. Spectral analysis of HRV at rest reveals two main components: 1) a high-frequency (HF) component in the range from 0.15 to 0.4 Hz, mainly due to respiratory sinus arrhythmia, and 2) a low-frequency (LF) component in the range from 0.04 to 0.15 Hz, which reflects both sympathetic and parasympathetic activity. Power in the HF band has been used as a measure of parasympathetic activity. Power in the LF band normalized by power in both the LF and HF bands has been considered a measure of sympathetic dominance. The ratio between the power in the LF and HF bands (LF/HF ratio) is considered a measure of sympathovagal balance [7].

Due to its relation with the ANS activity, HRV has been widely used to characterize the stress response. Most of the research on HRV response to stress is focused on the measurement of SNS excitation through the normalized power in the LF band and the sympathovagal ratio. A different approach is considered in [8], where respiratory sinus arrhythmia, as a marker of PNS tone, is proposed to assess stress and vulnerability to stress. In this approach, PNS tone is considered to parallel homeostasis and a withdrawal of PNS tone would represent the disruption of homeostasis induced by stress.

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TABLE I
PARAMETERS STUDIED IN THIS PAPER AND THEIR MEANING

Signal	Parameter	Meaning
HRV	d_{HRM}	Mean heart rate
	P_{VLF}	Power measured in the VLF band
	P_{LF}	Power measured in the LF band
	P_{HF}	Power measured in the HF band
	$P_{\mathrm{LF}\,n}$	Power normalized in the LF band
	$R_{\mathrm{LF/HF}}$	Ratio between LF and HF power
Respiration	F_R	Respiratory rate
	P_k	Peakness of the respiratory spectra
	N_k	Percentage of spectra used to estimate F_R

Most of the studies suggest higher sympathetic dominance during stress than during resting or relaxing conditions, however changes in specific HRV parameters published in the literature are inconsistent even when restricting to a specific emotional/cognitive type of stress. For example, an increase in the LF power has been reported during mental arithmetic [9]. An increase in the sympathovagal balance and in the normalized LF power during mental task was found in controls but not in patients with a prior myocardial infarction [10]. Mental stressors added during computer work caused a decrease in the HF power and an increase in the LF/HF ratio, but not an increase in the LF power in [11]. In [12] and [13], a decrease both in LF and HF powers is reported during mental load added to a normal office task. Lower HF power was also observed during the Stroop test and mental arithmetic, while LF power increased during the Stroop test and decreased during the arithmetic test [14].

Specific differences in stress stimulus and population are not enough to explain the differences found in the results. Some of the inconsistent results may be due to the methodology applied for the spectral analysis of HRV. Time-frequency analysis could allow us to characterize the nearly instantaneous response to acute stress, which may be blurred with time-invariant methods [15]. Moreover, differences in mean heart rate (HR) during stress and relaxing conditions can introduce a bias in HRV spectral parameters, which needs to be compensated for [16]. Finally, it has been shown that changes in the respiratory pattern alter the spectral content of HRV [9], [17], and mental stress was reported to alter the breathing pattern, increasing both the tidal volume and respiration rate [13], [18]. Respiratory variability and sigh rate also change during mental stress and attentional tasks [19]. Thus, stress-related changes in respiration may alter HRV parameters, obscuring their interpretation in terms of SNS and PNS activations [20].

In this paper, we analyze HRV and respiration changes in healthy subjects during acute emotional stress using time-frequency representations. Then, we include information on respiratory frequency in HRV analysis to obtain a more reliable interpretation of HRV parameters for stress assessment. A preliminary version of this paper has been reported [21], where the respiratory information was analyzed in a subset. As a glossary, the parameters and indices which are going to be studied in this paper are presented in Table I.

II. MATERIALS AND METHODS

A. Data Collection

A database of 46 volunteers (18 men and 28 women) with an age of 21.76 ± 4.48 years is used. These recordings were acquired in the Autonomous University of Barcelona (UAB) [22] and in the University of Zaragoza (UZ). The protocol defined in the following was approved by the Ethics Committee both at the UAB and UZ.

Each subject underwent a basal session and a stress session. These two sessions were completed in days close to each other and at the same hour (10 A.M. or 11.15 A.M., depending on the subject), trying to reproduce the same biorhythms-related stress conditions in both sessions. A chest-band-based respiratory signal and three orthogonal ECG leads, approximating the uncorrected bipolar X, Y, and Z leads, were continuously recorded with a sampling rate of 250 and 1000 Hz, respectively, using Medicom system (Medicom MTD Ltd., Russia).

The basal session (BL_B) consisted of a 35-min-length relaxing audition. The stress session tries to induce emotional stress by following a modification of the Trier Social Stress Test (TSST) [23]. This session included the following stages:

1) Baseline stage during stress session (BL_S): 10-min-length relaxing audition.
2) Story-telling stage (ST): three stories are told to the subject with a great amount of details. The subject is requested to remember as much details as possible, demanding a great amount of attention.
3) Memory task (MT): the subject is requested to tell back every remembered detail within 30 s for each story.
4) Stress anticipation (SA): subject is requested to wait for the evaluation of the memory test. The duration of this stage is 10 min.
5) Video exposition (VE): a projection of a video with the subject performance in the memory test is shown. The video showed twice each one of the three stories. First, an actor repeats the story in a perfect way, trying to make the subject believe that this is the common case. Subsequently, the subject (recorded during the MT stage) telling back the story is displayed.
6) Arithmetic task (AT): the subject has to count down from 1022 in steps of 13. In case of a calculation error, the subject is requested to restart from 1022. Although the subject is not expected to complete the countdown, he is requested to do so within 5 min. No subject completed the countdown.

The last five stages are considered stressful. BL_B, BL_S, and SA have longer duration that the other stages. Only the segment from 2 to 8 min (six central minutes of the first 10 min) was analyzed to avoid possible transient phenomena at the extrema of the stages, e.g., the own-day-stress level at the beginning and the possible boredom or expectation for the next stage of the subject.

B. Psychometric Evaluation

Psychometric evaluation applied to the whole sample was designed and it is used in this study as gold standard to know whether or not stress is induced. The following tests were used: perceived stress scale (PSS) measures the degree of overall stress in life situations of the subject [24]; visual analogue scale (VAS) to measure subjective stress in a numeric scale from 0 to 100; state-trait anxiety inventory (STAI), which is a commonly used measure of anxiety and it distinguishes between state anxiety (STAI_S), which is a temporary condition experienced in specific situations and trait anxiety (STAI_T), considered as a general tendency to perceive situations as threatening [25]. It is often used also in research as an indicator of distress [26], [27]. All tests were self-reported at the end of both basal and induced stress sessions.

C. Heart Rate Variability Analysis

First, heart beats are detected from Z lead of the recorded ECG signal using an algorithm based on wavelets [28]. Ectopic beats, missed and false detentions are identified [29]. From the beat occurrence time series using an algorithm based on the integral pulse frequency modulation model [16], which accounts for the presence of ectopic beats [29], an instantaneous HR signal $d_{\text{HR}}(n)$, sampled at 4 Hz, is obtained

$$d_{\text{HR}}(n) = \frac{1 + \mathfrak{m}(n)}{T(n)} \quad (1)$$

where $\mathfrak{m}(n)$ represents the modulating signal which carries the information from ANS and $T(n)$ is the mean HR, which is considered to be slow-time-variant by this model.

Then, a time-varying mean HR $d_{\text{HRM}}(n)$ is obtained by low-pass filtering $d_{\text{HR}}(n)$, with a cut-off frequency of 0.03 Hz:

$$d_{\text{HRM}}(n) = \frac{1}{T(n)}. \quad (2)$$

HRV signal is obtained as

$$d_{\text{HRV}}(n) = d_{\text{HR}}(n) - d_{\text{HRM}}(n). \quad (3)$$

Finally, the modulating signal is estimated as [16]

$$\mathfrak{m}(n) = \frac{d_{\text{HRV}}(n)}{d_{\text{HRM}}(n)}. \quad (4)$$

This modulating signal is supposed to carry the information of ANS activity without the influence of HR.

Time-frequency analysis is applied to $\mathfrak{m}(n)$ in order to characterize the rapid response of the ANS to stress. In this paper, the smooth pseudo Wigner–Ville distribution (SPWVD) is used since it provides a good compromise between interference terms reduction and a good time-frequency resolution, as well as an independent control of the time and frequency resolution [30], [31]. The SPWVD of $\mathfrak{m}(n)$, $P_{\mathfrak{M}}(n, m)$, is computed as

$$P_{\mathfrak{M}}(n,m) = 2 \cdot \sum_{l=-L+1}^{L-1} |h(l)|^2 \cdot \left[\sum_{n'=-N+1}^{N-1} g(n') a_{\mathfrak{M}} \times (n + n' + l) a_{\mathfrak{M}}{}^*(n + n' - l) \right] \cdot e^{-j2l(m/M)\pi}; m = -M+1, \ldots, M \quad (5)$$

where n and m are time and frequency indices. The analytic signal $a_{\mathfrak{M}}(n)$ is defined as $a_{\mathfrak{M}}(n) = \mathfrak{m}(n) + j \cdot \hat{\mathfrak{m}}(n)$, where $\hat{\mathfrak{m}}(n)$ represents the Hilbert transform of $\mathfrak{m}(n)$. The terms $g(n)$ and $h(l)$ are time and frequency smoothing windows, chosen to be Hamming windows whose lengths are $2 \cdot N + 1 = 203$ and $2 \cdot L + 1 = 1025$ samples, respectively [32].

Instantaneous power in classical LF (0.04–0.15 Hz) and HF (0.15–0.4 Hz) bands is computed from $P_{\mathfrak{M}}(n, m)$, yielding $P_{\text{LF}}(n)$ and $P_{\text{HF}}(n)$, respectively. Instantaneous power of the SPWVD of $d_{\text{HRM}}(n)$ is also computed and denoted $P_{\text{VLF}}(n)$. Instantaneous normalized LF power $P_{\text{LF}_n}(n) = P_{\text{LF}}(n)/(P_{\text{LF}}(n) + P_{\text{HF}}(n))$ and LF/HF ratio, $R_{\text{LF/HF}}(n) = P_{\text{LF}}(n)/P_{\text{HF}}(n)$ are also considered. The former analysis will be referred to as classical HRV.

D. Respiratory Rate Estimation

Respiration signal is band-pass filtered (cut-off frequencies of 0.03 and 0.9 Hz) and downsampled to 4 Hz.

Respiratory rate was estimated from this filtered respiratory signal by using an algorithm based on [33]. The method consists in the estimation of the respiratory frequency F_R from "peaked-conditioned" averaged spectra.

Every 5 s, a power spectrum density $S_k(f)$ is estimated by using the Welch periodogram from the kth 42 s length running window. Spectra obtained from 12 s-length subintervals overlapped 6 s are averaged. Subsequently, a measure of peakness is obtained from $S_k(f)$ as the percentage of power around the previous estimated respiratory rate $F_R(k-1)$ with respect to the total power within [0.08, 0.8 Hz] band:

$$P_k = \frac{\int_{F_R(k-1)-\delta}^{F_R(k-1)+\delta} S_k(f) df}{\int_{0.08}^{0.8} S_k(f) df} \cdot 100 \quad (6)$$

where δ value was empirically set as 0.1 Hz. Then, a peaked-conditioned average spectra $\bar{S}_k(f)$ is obtained by averaging those $S_k(f)$ which are sufficiently peaked:

$$\bar{S}_k(f) = \sum_{l=-L_s}^{L_s} \chi_{k-l} S_{k-l}(f) \quad (7)$$

where L_s was set to 2 in order to average a maximum of 5 spectra as in [33], and χ_{k-l} is a criterion to consider whether the power spectrum $S_{k-l}(f)$ is peaked enough or not:

$$\chi_k = \begin{cases} 1, & P_k \geq 65 \\ 0, & \text{otherwise} \end{cases} \quad (8)$$

allowing us to take part in the average only to those $S_k(f)$ whose P_k is above 65%.

Fig. 1 displays two spectra as examples, one with $P_k > 65\%$ (peaked enough to take part in the average), and another one with $P_k < 65\%$ (not peaked enough to take part in the average). Finally, the respiratory rate is estimated as the maximum of $\bar{S}_k(f)$ within the band [0.08, 0.8 Hz]:

$$F_R(k) = \arg\max_f \bar{S}_k(f); \quad f \in [0.08, 0.8 \text{ Hz}]. \quad (9)$$

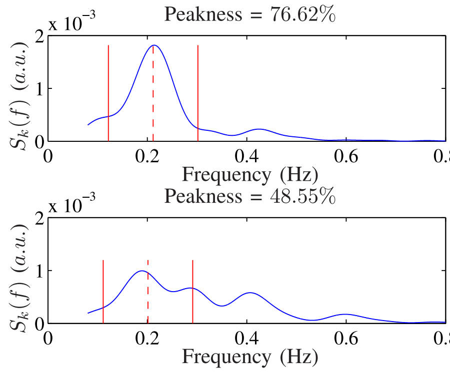

Fig. 1. Differences between spectra which satisfy the peakness condition and those which do not. Red lines illustrate the limits of the integrating interval of the numerator in P_k and the dashed line marks the previous respiratory rate estimated $F_R(k-1)$ [see (6)].

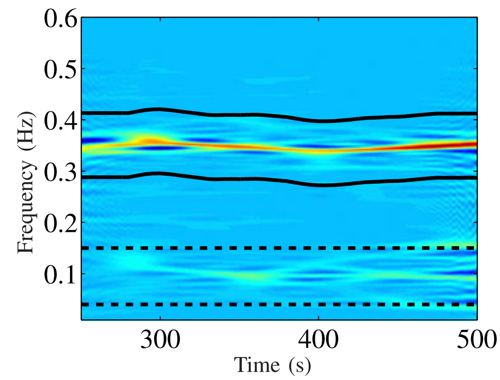

Fig. 2. $P_{\mathfrak{M}}(n,m)$ for one suject. Solid black lines represent $\Omega_{\mathrm{HF}_R}(n)$. Dashed black lines represent the LF band.

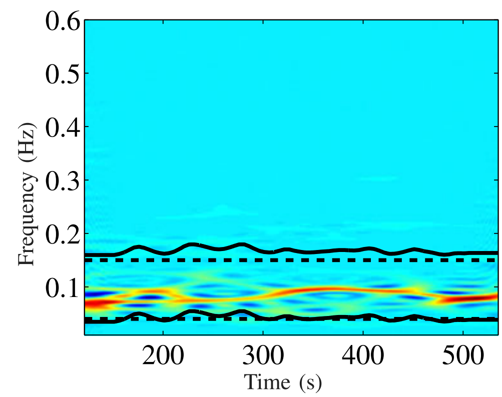

Fig. 3. $P_{\mathfrak{M}}(n,m)$ for one suject, representing the overlap between the two bands because of a low respiratory rate. Solid black lines represent $\Omega_{\mathrm{HF}_R}(n)$. Dashed black lines represent the LF band.

Studied parameters were respiratory rate $F_R(k)$, the peakness P_k, and the percentage of spectra which take part in the peaked-conditioned average N_k, considering the last two parameters to be related to the respiratory stability. Note that it may occur that no spectrum is peaked enough at some time instants. In those cases, respiratory parameters are not studied. Respiratory rate could not be estimated during MT and AT stages since speech modifies the respiratory pattern and no spectra would satisfy the peakness criterium.

E. HRV Study Including Respiratory Information

Analysis of respiration revealed changes in the respiratory frequency during stress conditions with respect to relax [18], [19]. In order to obtain a more reliable assessment of the PNS activity, respiratory frequency estimation is included in HRV analysis redefining the HF band centered at respiratory frequency as in [34]. The method described in Section II-D offers an estimation of respiratory rate every 5 s, so a linear interpolation is made in order to obtain a respiratory frequency signal $F_R(n)$ with the same sampling rate than the HRV series (4 Hz).

The VLF and LF bands are the classical bands used in Section II-C ([0, 0.04 Hz] and [0.04, 0.15 Hz], respectively), while the HF band this time is defined as

$$\Omega_{\mathrm{HF}_R}(n) = [F_R(n) - 0.05\ \mathrm{Hz}, F_R(n) + 0.05\ \mathrm{Hz}]. \quad (10)$$

The choice of ± 0.05 Hz is done to make this bandwidth comparable to the LF one. In Fig. 2, the new localization of the HF band centered in the respiratory rate can be seen.

In some stages of the test, especially during the basal stage, a low respiratory rate makes $\Omega_{\mathrm{HF}_R}(n)$ overlap with the LF band. In order to avoid the measurement of the same power in both bands, a threshold that delimits the amount of overlapping percentage between $\Omega_{\mathrm{HF}_R}(n)$ and the LF band is defined. If at a given time instant n, overlapping is higher than experimentally adjusted 50%, power in those bands at that instant are not computed. Fig. 3 shows an example where the respiratory rate (mean respiratory rate is 0.1041 Hz) is within the LF band (inside dashed black lines), so $\Omega_{\mathrm{HF}_R}(n)$ (between solid black lines) overlaps with it. The percentage of overlapping is higher than the fixed threshold (50%) during the whole interval displayed, so power in LF and HF bands are not computed for any time instant within this interval.

In this paper, the inclusion of the respiratory rate information in HRV analysis comprises two steps: 1) exclusion from the analysis of cases where respiratory rate falls within the LF band, and 2) redefinition of the HF band centered at respiratory frequency. In order to assess the improvement of each step, the following analysis is done. First, classical HRV indices described in Section II-C are studied only in a subset after expurgation of cases where respiratory rate overlaps with the LF band. This analysis is denoted as HRV_E. Second, in the expurgated subset, the HF band is redefined centered at the respiratory rate. This analysis is denoted as $\mathrm{HRV}_E^{\Omega_{\mathrm{HF}_R}}$.

F. Statistical Analysis

About the psychometric tests, in PSS, VAS, and STAI (in both phases, state and trait), a paired Wilcoxon statistical test is applied between the responses of all our subjects the first day (basal session) and those the second day (stress session) in order to identify changes related to stress.

TABLE II
MEDIAN ± MAD OF SELECTED PSYCHOMETRIC TESTS IN BOTH SESSIONS

Test	Basal session	Stress session
PSS	20 ± 2.4	20 ± 2.5
STAI$_S$	12 ± 6.1	22.5 ± 9.8 ***
STAI$_T$	18 ± 7.4	19 ± 7.7
VASS	30 ± 18.6	50 ± 17.6 ***

p-values: *($p < 0.05$); **($p < 0.01$); ***($p < 0.001$)

Intrasubject mean of each studied HRV index was obtained for each stage of the protocol: $\bar{d}_{\mathrm{HRM}}$, $\bar{P}_{\mathrm{VLF}}$, $\bar{P}_{\mathrm{LF}}$, $\bar{P}_{\mathrm{HF}}$, $\bar{P}_{\mathrm{LF}_n}$, and $\bar{R}_{\mathrm{LF/HF}}$. In addition, three respiratory parameters are studied too: the intrasubject median of respiratory rate $\bar{F}_R$, the peakness measure $\bar{P}_k$, and the percentage of spectra used to compute the peaked-conditioned averaging $\bar{N}_k$. In order to study if there are significant differences between the studied indices computed at two stages, the paired Wilcoxon statistical test was performed. Since such a test requires subjects having measures in both stages, the number of studied subjects varies depending on the stages being compared. Comparisons were done for the three different strategies of analysis considered: (HRV, HRV$_E$, and HRV$_E^{\Omega_{\mathrm{HF}_R}}$). For both statistical tests with the three strategies used, the p-value threshold adopted to define significance is 0.05.

Furthermore, the area under receiver operating characteristics curve (AUC) for each of the indices described in this paper was calculated to evaluate their capacity to discriminate between relax (grouping BL_B and BL_S) and stress (grouping ST, SA, and VE) stages, all of them evaluated over the expurgated dataset, so allowing results comparison.

III. RESULTS

A. Psychometric Evaluation

Table II shows median and median absolute deviation (MAD) [35] of the three selected psychometric test across sessions described in Section II-B. The STAI$_T$ subscale does not change, but STAI$_S$ subscale increases significantly across sessions (Wilcoxon test $p < 0.001$). Similarly, significant differences were obtained when the VAS scale to measure subjective stress is considered (Wilcoxon test $p < 0.001$).

B. Respiratory Parameters

As mentioned before, respiratory rate could not be estimated in all the subjects from all stages of the stress session. This occurred in 4 subjects out of 46 (8.7%) in BL_S; 4 (8.7%) in ST; 5 (10.9%) in SA; and 3 (6.5%) in VE.

One example of respiratory estimation performance is presented in Fig. 4, where differences between BL_S and ST stages are shown, evidencing higher and less stable respiratory rate during ST than during BL_S.

Table III shows the intersubject median and MAD of $\bar{F}_R$, $\bar{P}_k$, and $\bar{N}_k$ among all the subjects. The respiratory rate is observed to be higher and less stable (lower $\bar{P}_k$ and $\bar{N}_k$) during the stress stages than during the BL_S stage. Table III also shows results of the paired Wilcoxon test. The number of subjects in each comparison are: 38 subjects in BL_S versus ST; 37 in BL_S versus SA and ST versus SA; and 39 in BL_S versus VE, ST versus VE, and SA versus VE.

When comparing both basal stages (BL_{B} versus BL_S) for all the subjects, results show a similar respiratory rate (0.21 ± 0.06 Hz versus 0.23 ± 0.06 Hz) and percentage of spectra used ($79.2 \pm 5.5\%$ versus $79.6 \pm 6.1\%$). The peakness is slightly lower in BL_B ($83.6 \pm 7.2\%$ versus $87.9 \pm 12.0\%$). These differences are not statistically significant according with the paired Wilcoxon test (with 30 subjects).

C. HRV Parameters

One example of the instantaneous HR signal $d_{\mathrm{HR}}(n)$, the modulating signal $\mathfrak{m}(n)$, and its SPWVD $P_{\mathfrak{m}}(n, m)$ is displayed in Fig. 5 for a subject during 1 min in stages BL_S and ST. The variation of HF band centered at respiratory rate can be appreciated in the SPWVD, with a low respiratory rate that overlaps with the LF band in BL_S and just the opposite in ST, showing values over the limit of the HF classical band (0.4 Hz).

The number of excluded subjects due to LF band overlapping with $\Omega_{\mathrm{HF}_R}(n)$ is 9 out of 42 (21.4%) in BL_S and no one in the other stages. Table IV shows the intersubject median and MAD of the HRV indices with the three different analysis (HRV, HRV$_E$, and HRV$_E^{\Omega_{\mathrm{HF}_R}}$). To facilitate their interpretation, results from $\bar{P}_{\mathrm{HF}}$, $\bar{P}_{\mathrm{LF}_n}$, and $\bar{R}_{\mathrm{LF/HF}}$ are presented in a boxplot mode in Fig. 6, for the three parameters which result more affected by the proposed test. The resulting number of subjects used in the paired comparisons for HRV$_E$ and HRV$_E^{\Omega_{\mathrm{HF}_R}}$ analysis are 30 in BL_S versus ST, BL_S versus SA, and BL_S versus VE; 37 in ST versus SA; and 39 in ST versus VE and SA versus VE.

Note that results of $\bar{d}_{\mathrm{HRM}}$, $\bar{P}_{\mathrm{VLF}}$, and $\bar{P}_{\mathrm{LF}}$ are the same in the analysis HRV$_E$ and HRV$_E^{\Omega_{\mathrm{HF}_R}}$, because they are measured over the same subjects. Relevant to note is the percentage of subjects with a respiratory rate higher than 0.35 Hz (so part of $\Omega_{\mathrm{HF}_R}(n)$ is over 0.4 Hz, upper limit of the classical HF band): 4.7% in BL_S (2 out of 42); 47.6% in ST (20 out of 42); 19.5% in SA (8 out of 41); and 30.2% in VE (13 out of 43). This fact leads to the differences obtained in $\bar{P}_{\mathrm{HF}}$, $\bar{P}_{\mathrm{LF}_n}$, and $\bar{R}_{\mathrm{LF/HF}}$ between HRV$_E$ and HRV$_E^{\Omega_{\mathrm{HF}_R}}$.

Differences between the two basal stages of both sessions are only evaluated using HRV$_E^{\Omega_{\mathrm{HF}_R}}$ analysis. Results for BL_S are already presented in Table IV and for BL_{B} they are: $\bar{d}_{\mathrm{HRM}} = 1.26 \pm 0.17(\mathrm{s}^{-1})$; $\bar{P}_{\mathrm{VLF}} = 0.51 \pm 0.13(\mathrm{s}^{-2})$; $\bar{P}_{\mathrm{LF}} \cdot 10^3 = 1.38 \pm 1.12(\mathrm{ad})$; $\bar{P}_{\mathrm{HF}} \cdot 10^3 = 0.81 \pm 1.19(\mathrm{ad})$, $\bar{P}_{\mathrm{LF}_n} = 0.61 \pm 0.11(\mathrm{nu})$ and $\bar{R}_{\mathrm{LF/HF}} = 1.6 \pm 0.73(\mathrm{nu})$. An increase in the last four parameters is observed, although not statistically significant (paired Wilcoxon test over 26 suitable subjects).

Table V shows the AUC for HRV indices and respiratory parameters in the original dataset and for HRV$_E^{\Omega_{\mathrm{HF}_R}}$ in the expurgated dataset for discriminating between the grouped relax and stress sets (defined in Section II-F). Notice that respiratory parameters have worse behavior with the expurgated dataset

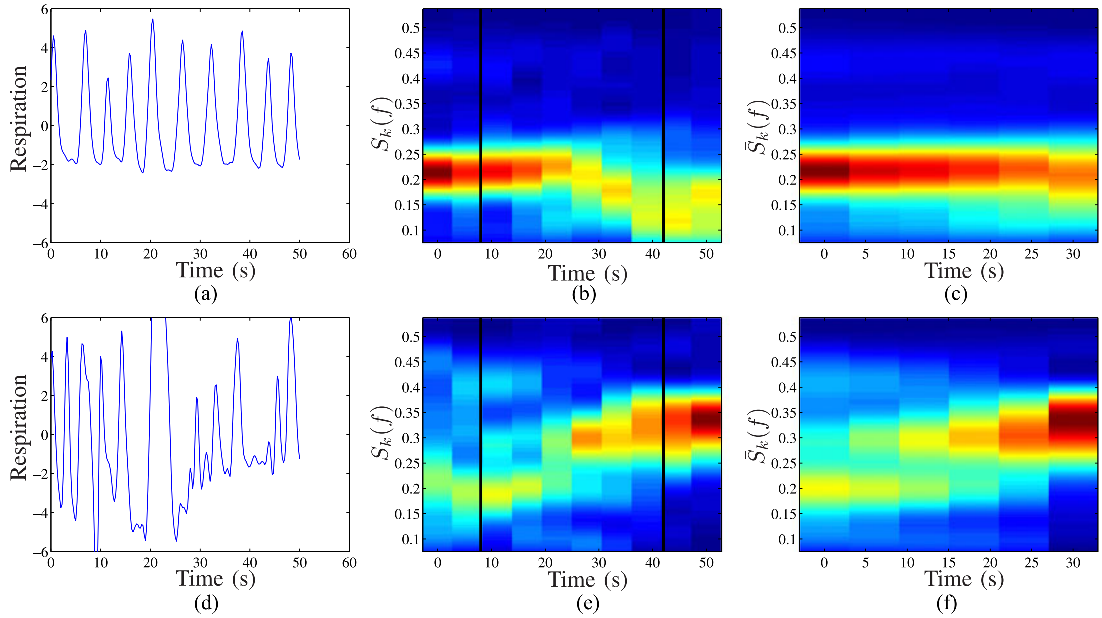

Fig. 4. (a) and (d) respiratory signal; (b) and (e) $S_k(f)$; (c) and (f) $\bar{S}_k(f)$; in basal stage (above) and story telling (below). Black lines in $S_k(f)$ delimits the time interval showed in $\bar{S}_k(f)$.

TABLE III
MEDIAN ± MAD OF $\bar{F}_R$, $\bar{P}_k$ AND $\bar{N}_k$ FROM ALL SUBJECTS IN THE DIFFERENT PARTS OF THE TEST

Stage	BL_S	ST		SA		VE	
$\bar{F}_R\,(Hz)$	0.23 ± 0.06	0.35 ± 0.05	***	0.29 ± 0.05	*** †††	0.32 ± 0.04	*** ††† ⋄⋄⋄
$\bar{P}_k\,(\%)$	79.74 ± 6.06	78.81 ± 8.22		71.71 ± 8.08	*** †††	72.55 ± 7.06	***
$\bar{N}_k\,(\%)$	87.94 ± 12.01	54.54 ± 11.87	***	76.57 ± 10.61	*** †††	74.37 ± 9.17	*** †††

Statistical differences are represented by: *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$) when compared with BL_S; similar for † when compared with ST and for ⋄ with SA.

because lower respiratory rate are discarded and most of them belongs to relax set.

IV. DISCUSSION

In this paper, respiration and HRV are analyzed during different stress levels.

Changes across sessions in psychometric scores measuring stress show that the chosen protocol to induce stress, the modified TSST, is useful for stress generation. As expected, our results showed that the STAI_T and the PSS are not affected by the TSST. In this sense, a trait, considered a stable, general tendency to perceive many situations as threatening should not be affected by changes in a particular situation (i.e., our experimental conditions) [25]; similarly, the PSS is a more stable measure of stress. On the contrary, the TSST may produce a temporal perception of the experimental conditions as a threat. Statistically significant changes in the stress scores obtained in the STAI_S, and in the VAS tend to confirm that our experimental protocol have produced measurable stress in our participants (see Table II).

Respiratory rate was significantly higher (according to the paired Wilcoxon test) during stressful stages than during the relax stage, in agreement with the results reported in [9], where the baseline recording presented a lower respiratory rate than in attention or mental arithmetic task.

Spectral peakness and the percentage of spectra accepted to compute the respiratory rate are studied in this paper as measures of respiration stability. The more the stable respiration is the more peaked the spectra are and a higher number of them are included in the average. Results obtained for both parameters show significantly more stable respiration during relax than during the stress stages of the protocol. The less peaked spectra are found in VE, while the lowest percentage of accepted spectra is in ST. In [19], respiratory variation measured as the variation of a breath component over a sampling period of 15 min is computed as a coefficient that increased with mental task compared to relax situation.

Inclusion of respiratory rate information in HRV analysis has been proposed in this study for stress assessment. First, those subjects with respiratory rate laying over the LF band were expurgated. Second, the HF band was redefined based on the respiratory rate. The reason why the overlapping happens is that the respiratory rate is variable and depends on the stage and the type of task being performed. As the respiratory rate is lower in basal stages, in some cases, these segments are prone to suffering from overlapping of the new defined HF band centered at the respiratory rate with the fixed LF band. The choice of 50% overlap as a threshold is an arbitrary compromise between not discarding lots of signals and still measuring

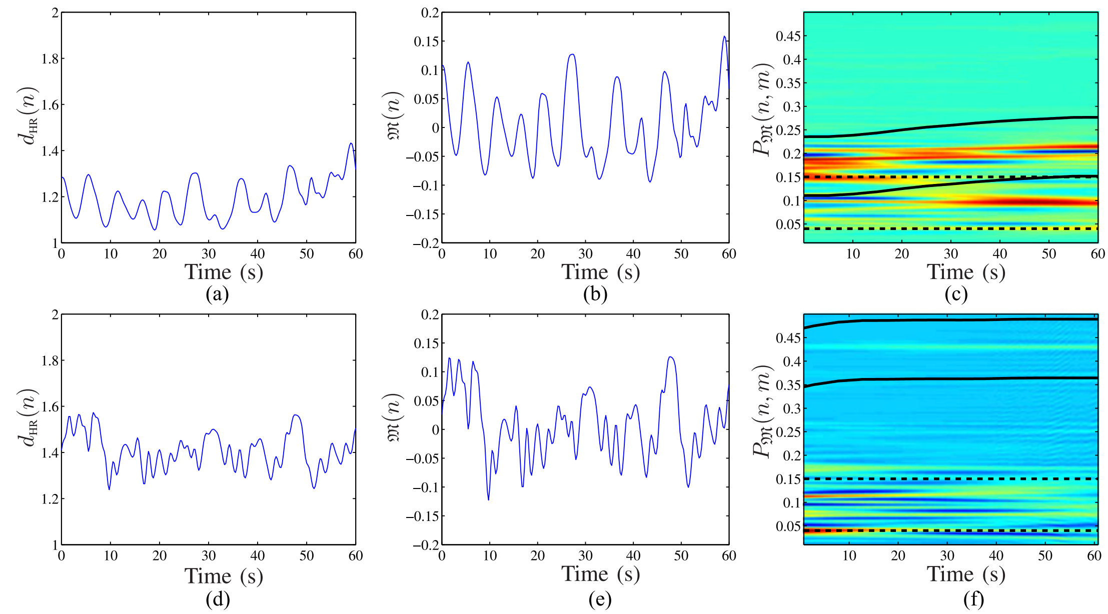

Fig. 5. (a) and (d) $d_{\rm HR}(n)$; (b) and (e) $\mathfrak{m}(n)$ signals; (c) and (f) its respective SPVWD in a time-frequency map showing the variations of HF band related to respiration in pre-relaxing stage (above) and story telling (below).

TABLE IV

MEDIAN ± MAD OF $\bar{d}_{\rm HRM}$, $\bar{P}_{\rm VLF}$, $\bar{P}_{\rm LF}$, $\bar{P}_{\rm HF}$, $\bar{P}_{{\rm LF}_n}$, $\bar{R}_{\rm LF/HF}$ FROM ALL SUBJECTS IN THE DIFFERENT PARTS OF THE TEST WITH PARAMETERS MEASURED WITH THE THREE CASES EXPLAINED (HRV, HRV$_E$, AND HRV$_E^{\Omega_{{\rm HF}_R}}$)

Stage	Analysis	$\bar{d}_{\rm HRM}(s^{-1})$	$\bar{P}_{\rm VLF}(s^{-2})$	$\bar{P}_{\rm LF}(ad)$	$\bar{P}_{\rm HF}(ad)$	$\bar{P}_{{\rm LF_n}}(nu)$	$\bar{R}_{\rm LF/HF}(nu)$
BL_S	HRV	1.20 ±0.15	0.49 ±0.12	1.67 ±2.40	1.17 ±1.92	0.50 ±0.18	1.45 ±2.16
	HRV_E	1.21 ±0.15	0.50 ±0.12	1.33 ±1.03	1.18 ±1.95	0.53 ±0.14	1.15 ±0.82
	$HRV_E^{\Omega_{{\rm HF}_R}}$				0.79 ±1.28	0.58 ±0.17	1.40 ±1.62
ST	HRV	1.40 ±0.22 ***	0.61 ±0.21 ***	2.01 ±1.11	0.86 ±0.84	0.68 ±0.11 **	2.15 ±1.50
	HRV_E	1.40 ±0.22 ***	0.61 ±0.22 ***	2.13 ±1.11	0.85 ±0.89	0.68 ±0.12 ***	2.15 ±1.35 ***
	$HRV_E^{\Omega_{{\rm HF}_R}}$				0.38 ±0.59 **	0.83 ±0.13 ***	5.01 ±5.04 ***
SA	HRV	1.23 ±0.15 † † †	0.49 ±0.11 † † †	1.96 ±2.37	1.15 ±1.97 †	0.65 ±0.14 ††	1.83 ±0.93 ††
	HRV_E	1.24 ±0.14 * † † †	0.49 ±0.11 * † † †	1.87 ±2.51 ***	1.10 ±2.15 †	0.62 ±0.14 ** ††	1.61 ±1.26 ** ††
	$HRV_E^{\Omega_{{\rm HF}_R}}$				0.72 ±1.34 ††	0.71 ±0.15 ** † † †	2.44 ±3.97 ** ††
VE	HRV	1.27 ±0.17 † † † ⋄⋄	0.50 ±0.15 † † † ⋄	1.43 ±2.14 * † ⋄ ⋄ ⋄	1.05 ±1.72	0.58 ±0.15 † † † ⋄	1.41 ±0.94 † † † ⋄
	HRV_E	1.26 ±0.17 † † † ⋄⋄	0.50 ±0.15 † † † ⋄	1.25 ±2.19 †† ⋄ ⋄ ⋄	0.93 ±1.83	0.57 ±0.15 † † † ⋄	1.34 ±1.07 † † † ⋄
	$HRV_E^{\Omega_{{\rm HF}_R}}$				0.56 ±1.01 †† ⋄	0.68 ±0.15 † † †	2.14 ±3.07 † † †

The measure unit are seconds (s), adimensional (ad), and normalized units (nu). Statistical differences are represented by: *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$) when compared with BL_S; similar for † when compared with ST and for ⋄ with SA.

power mostly related to the selected band. Note that this 50% threshold is equivalent to impose a restriction in the respiratory rate, which has to be outside the LF band (i.e., higher than 0.15 Hz). When this threshold is exceeded, this stage is discarded because it cannot be assured that the power measured in either band represents a mainly sympathetic or parasympathetic activity. However, these segments cannot be discarded in a real stress recognition task and consequently further studies are required to handle this overlapping issue in real applications.

Classical HRV analysis showed significant differences with respect to the BL_S stage: $\bar{d}_{\rm HRM}$, $\bar{P}_{\rm VLF}$, and $\bar{P}_{{\rm LF}_n}$ increases in ST, $\bar{P}_{\rm LF}$ decreases in VE, and $\bar{P}_{\rm HF}$ decreases in ST. The

TABLE V
AUC OF $\bar{F}_R$, $\bar{P}_k$, $\bar{N}_k$, $\bar{d}_{\mathrm{HRM}}$, $\bar{P}_{\mathrm{VLF}}$, $\bar{P}_{\mathrm{LF}}$, $\bar{P}_{\mathrm{HF}}$, $\bar{P}_{\mathrm{LF}_n}$, AND $\bar{R}_{\mathrm{LF/HF}}$

Parameter	AUC for HRV (%)	AUC for HRV_{E}(%)	AUC for $HRV_{\mathrm{E}}^{\Omega_{\mathrm{HF_R}}}$(%)
$\bar{F}_{\mathrm{R}}$	88.2	81.8	
$\bar{P}_{\mathrm{k}}$	67.5	67.1	
$\bar{N}_{\mathrm{k}}$	80.8	81.2	
$\bar{d}_{\mathrm{HRM}}$	60.3	59.2	
$\bar{P}_{\mathrm{VLF}}$	57.9	58.1	
$\bar{P}_{\mathrm{LF}}$	51.6	61.1	
$\bar{P}_{\mathrm{HF}}$	54.2	56.6	63.9
$\bar{P}_{\mathrm{LF_n}}$	53.7	64.9	69.4
$\bar{R}_{\mathrm{LF/HF}}$	53.7	64.9	69.4

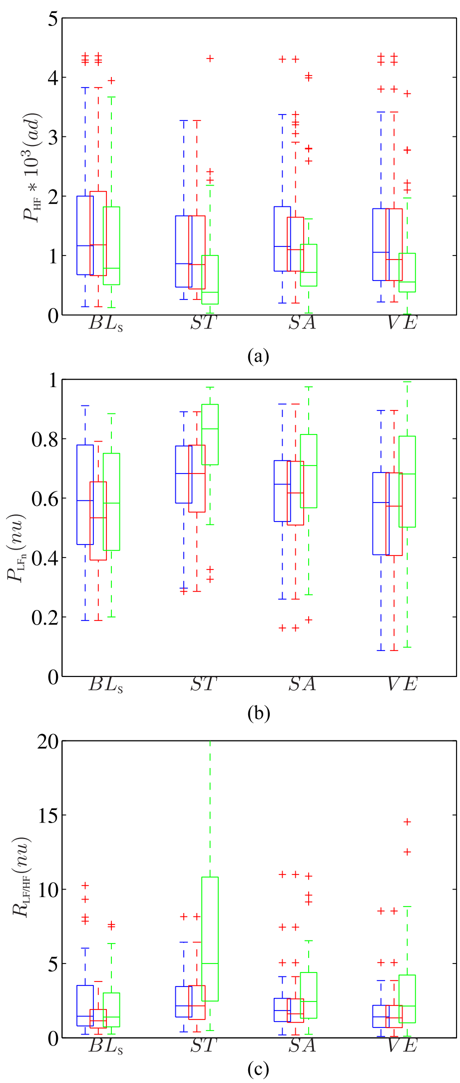

Fig. 6. (a) Boxplot of the power in HF band, (b) the normalized power in LF band, and (c) the ratio between LF and HF powers for all the stages using the three analysis: HRV (in blue); HRV$_E$ (in red), and HRV$_E^{\Omega_{\mathrm{HF}_R}}$ (in green).

decrease of $\bar{P}_{\mathrm{LF}}$ in VE with respect to BL_S is related to the fact that in some cases respiration lays in LF band during relax, leading to an overestimation of $\bar{P}_{\mathrm{LF}}$ in BL_S.

When expurgating the dataset to account for respiratory rate inclusion in HRV, we observed that now $\bar{P}_{\mathrm{LF}}$ increases significantly in SA while no significant differences are observed in VE. On the other hand, $\bar{P}_{\mathrm{HF}}$ now does not significantly change for any stress stage with respect to BL_S. $\bar{P}_{\mathrm{LF}_n}$ and $\bar{R}_{\mathrm{LF/HF}}$ also increases significantly for ST and SA. Again, it is necessary to mention that this expurgation is made for the statistical study of HRV including respiration parameters in this paper. In practice, the added value of this imbricated analysis cannot be used for the subjects that do overlap and so for those cases a decision can be made based just on respiratory parameters.

Furthermore, it may also happen that the respiratory rate is above the classical HF band during stress, as exemplified in Fig. 2, leading to an underestimation of $\bar{P}_{\mathrm{HF}}$ and, consequently, an overestimation of $\bar{P}_{\mathrm{LF}_n}$ and $\bar{R}_{\mathrm{LF/HF}}$. According to this observation, the HF band has been redefined based on the respiratory rate in the expurgated dataset. The bandwidth used in this new band is 0.1 Hz, similar to the LF band bandwidth and smaller than the one used in the classical HF band. This selection is made in order to avoid exaggerated overlapping between the LF band and the new HF band. Comparing powers in both HF bands (classical one and with the new defined limits), 66.8% of the power in the classical band is measured with the 0.1 Hz bandwidth, although that the pairwise difference between data has not a mean equal to zero ($p < 0.001$ in the paired Student test). Notice that 0.1 respect to 0.25 (classical bandwidth) is only the 40% of the total area, so this new bandwidth still expresses most of the spectral power, being then wise to use it as parasympathetic quantification without much loss, as corroborated by the study results. Now, additionally to previous observations, $\bar{P}_{\mathrm{HF}}$ decreases significantly in ST with respect to BL_S.

Some works have reported an increase in the LF band during stress [9], [10] while others have not found significant differences [11]. In our study, an increase is observed in $\bar{P}_{\mathrm{LF}}$ during ST and SA with respect to BL_S, being statistically significant during SA.

$\bar{P}_{\mathrm{HF}}$ is lower in all stages using the HRV$_E^{\Omega_{\mathrm{HF}_R}}$ analysis, since the HF band is narrower. However, it is appreciated a larger relative reduction during stress stages with respect to BL_S in $\bar{P}_{\mathrm{HF}}$ with the HRV$_E^{\Omega_{\mathrm{HF}_R}}$ analysis than in the other two (HRV and HRV$_E$), supporting the use of respiratory sinus arrhythmia to assess stress, as proposed in [8] and confirmed by [11]–[13]. The larger decrease of $\bar{P}_{\mathrm{HF}}$ in ST than in SA may be related to the different types of stressors. For example, during ST, there is a large demand of attention, while during SA the stress is mainly psychological or emotional.

$\bar{P}_{\mathrm{LF}_n}$ and $\bar{R}_{\mathrm{LF/HF}}$ are significantly higher during ST and SA than during BL_S, including respiratory information, suggesting a sympathetic dominance. These results are in agreement with those reported in [9]–[11], [17]. Note that $\bar{P}_{\mathrm{LF}_n}$ and $\bar{R}_{\mathrm{LF/HF}}$, with the classical analysis without the use of respiratory information, did not show significant differences.

The former findings obtained when the respiratory rate is included in HRV analysis are in line with the physiological bases of stress, like activation of sympathetic and withdrawal of parasympathetic stimulation. Previous classical HRV analysis did not allow these physiological interpretations, supporting the adequateness of respiratory rate inclusion in HRV analysis.

In this study, the overestimation of $\bar{P}_{\mathrm{LF}}$ and underestimation of $\bar{P}_{\mathrm{HF}}$ due to a low respiratory rate are avoided by expurgating those subjects where respiration is in the LF band. However, for those subjects in need for expurgation with this analysis, further studies should address alternatives to separate parasympathetic and sympathetic activities in this situation.

Indices related to sympathetic dominance ($\bar{P}_{\mathrm{LF}_n}$ and $\bar{R}_{\mathrm{LF/HF}}$) got the best results among HRV indices in terms of AUC values for discrimination between relax and stress. However, respiratory parameters presented higher discrimination power than any HRV index, suggesting its potential for stress assessment.

One limitation of the study is that the method is only valid for those intervals when the respiratory rate can be properly estimated (sufficiently peaked spectra). However, it is not suitable to estimate the respiratory rate in the stages where the subject is speaking. In this situation, respiration has a broadband spectrum [36], [37] where it is not possible to find a dominant peak related to the respiratory rate and, subsequently, analyze respiratory parameters or include the respiratory rate in HRV analysis.

The complementary information that HRV analysis can add to respiration analysis for stress assessment should be considered in a larger study and should include those cases where the respiratory rate cannot be estimated robustly.

V. Conclusion

Frequency domain HRV indices, computed in classical terms, scarcely show statistical differences during stress. When respiratory rate information is used to guide HRV analysis, it allows us to avoid the overestimation of sympathetic activity and the underestimation of parasympathetic activity that occurs when the respiration rate lies in the LF band, as well as the underestimation of parasympathetic activity when the respiratory frequency is above 0.35 Hz. This combined HRV and respiratory rate analysis increases the statistical differences among different stress situations, where a major sympathetic dominance is observed. Finally, results showed that considering just respiratory rate information, a higher discriminative power, understanding discriminative power as the potential of the index to discriminate between relax and stress stages, is obtained, suggesting that the respiratory rate can also discriminate the different stress states. This, however, comes at the cost of losing the excerpts where this rate cannot be estimated.

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