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Heart-brain interactions during social and cognitive stress in hypertensive disease: a multidimensional approach

Running Title: Multidimensional markers of stress in hypertension

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Abstract

Hypertensive disease (HTD), a prominent risk factor for cardiovascular and cerebrovascular diseases, is characterized by elevated stress-proneness. Since stress levels are underpinned by both cardiac and neural factors, multidimensional insights are required to robustly understand their disruption in HTD. Yet, despite their crucial relevance, heart rate variability (HRV) and multimodal neurocognitive markers of stress in HTD remain controversial and unexplored, respectively. To bridge this gap, we studied cardiodynamic as well as electrophysiological and neuroanatomical measures of stress in HTD patients and healthy controls. Both groups performed the Trier Social Stress Test (TSST), a validated stress-inducing task comprising a baseline and a mental stress period. During both stages, we assessed a sensitive HRV parameter (the low frequency/high frequency [LF/HF ratio]) and an online neurophysiological measure (the heartbeat-evoked potential [HEP]). Also, we obtained neuroanatomical data via voxel-based morphometry (VBM) for correlation with online markers. Relative to controls, HTD patients exhibited increased LF/HF ratio and greater HEP modulations during baseline, reduced changes between baseline and stress periods, and lack of significant stress-related HRV modulations associated with the grey matter volume of putative frontrostriatal regions. Briefly, HTD patients presented signs of stress-related autonomic imbalance, reflected in a potential basal stress overload and a lack of responsiveness to acute psychosocial stress, accompanied by neurophysiological and neuroanatomical alterations. These multimodal insights underscore the relevance of neurocognitive data for developing innovations in the characterization, prognosis, and treatment of

HTD and other conditions with autonomic imbalance. More generally, these findings may offer new insights into heart-brain interactions.

Introduction

Hypertensive disease (HTD), defined by high blood pressure (Unger *et al.*, 2020), ranks among the highest risk factors for disabling cardiovascular and cerebrovascular diseases (Ruediger *et al.*, 2004; Thayer *et al.*, 2010). Moreover, this disorder is typified by elevated stress-proneness (Wirtz *et al.*, 2006; Liu *et al.*, 2017a), a factor that also undermines cardiovascular function (Cohen *et al.*, 2007; McEwen, 2007) and further compromises the patients' physical and mental health (Wiener *et al.*, 2020). Since stress levels are underpinned by both cardiac and neural mechanisms (Gianaros & Sheu, 2009; Allen *et al.*, 2014), multidimensional insights are required to robustly understand their disruption in HTD. Measures of heart rate variability (HRV) are robust markers of mental stress (Taelman *et al.*, 2009; Allen *et al.*, 2014; Kim *et al.*, 2018), especially if complemented with neurocognitive assessments (MacKinnon *et al.*, 2013; Wei *et al.*, 2018). However, HRV studies on HTD are controversial due to methodological inconsistencies and limitations (including sub-optimal methods to induce stress, unreliable markers of autonomic activity, and inconsistent HRV measures). Here, we administered the Trier Social Stress Test (TSST), a validated stress-inducing task (Kirschbaum *et al.*, 1993; Allen *et al.*, 2017), to HTD patients and healthy controls, targeting a highly sensitive HRV parameter –i.e., the low frequency/high frequency (LF/HF) ratio– as well as electrophysiological and neuroimaging markers of heart-brain interactions.

Autonomic responses to induced mental stress are reliably indexed by HRV modulations (Taelman *et al.*, 2009; Castaldo *et al.*, 2015; Kim *et al.*, 2018). Compared to other stress measures, HRV offers a relatively simple, non-invasive approach which has been increasingly used to assess autonomic function in various populations and paradigms (Thayer & Sternberg, 2006; Vlcek *et al.*, 2008; Chandola *et al.*, 2010). Specifically, the LF/HF ratio outperforms single frequency-domain components in capturing the sympathovagal balance (Pagani *et al.*, 1984; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Vlcek *et al.*, 2008; Goldstein *et al.*, 2011) during acute psychological stress (Xhyheri *et al.*, 2012; Allen *et al.*, 2014). In HTD patients, autonomic imbalance involves increased sympathetic and decreased parasympathetic activity at rest (Langewitz *et al.*, 1994; Madsen *et al.*, 2008; Thayer *et al.*, 2010), alongside increased sympathetic reactivity (Kaushik *et*

al., 2004; Wirtz *et al.*, 2006) and lack of significant modulations of the LF/HF ratio (Garafova *et al.*, 2014) during stress; all aligned with an impaired responsiveness to autonomic demands (Madsen *et al.*, 2008). However, few studies have targeted the latter variable and most have favored sub-optimal mental stress inductors, such as the Stroop test (Garafova *et al.*, 2014) or mental arithmetic paradigms (Ruediger *et al.*, 2004), i.e., purely cognitive (non-socially-laden) tasks considered to induce small or inconsistent effects in isolation (Allen *et al.*, 2014).

Furthermore, although studies that concurrently examined brain markers and peripheral measures in HTD have been encouraged (Jennings & Zanstra, 2009), no previous report seems to have explored multidimensional brain correlates of stress in HTD. In particular, the use of a task indexed with structural and temporal brain measures may reveal different spatiotemporal mechanisms (Melloni *et al.*, 2015; García-Cordero *et al.*, 2016; Melloni *et al.*, 2016; Ibáñez, 2018; Fittipaldi *et al.*, 2020) underlying stress alterations, paving the way for new insights to inform the characterization and future treatments of this disease (Devor *et al.*, 2013). To this end, we assessed ongoing modulations of the heartbeat evoked potential (HEP), an automatic cortical measure of interoceptive cardiac signals (Schandry & Montoya, 1996; Pollatos *et al.*, 2005) modulated by stress levels in healthy subjects (Schulz *et al.*, 2013) and cardiac patients (Gray *et al.*, 2007). Moreover, HEP modulations have been linked to HRV in normotensives (MacKinnon *et al.*, 2013). Crucially, this neurophysiological marker is disrupted in HTD patients (Yoris *et al.*, 2018), suggesting that sensing of autonomic visceral and vascular body signals (Craig, 2002; Critchley & Harrison, 2013; Salamone *et al.*, 2020) is associated with peripheral cardiovascular disruptions. This might reflect altered allostasis, that is, neural predictions of interoceptive signals to regulate the body's physiological system and support psychological phenomena including recognition of and reaction to psychosocial stress (Kleckner *et al.*, 2017; Mocayar Marón *et al.*, 2019). Also, from a neuroanatomical perspective, stress-induced HRV has been related to higher grey matter volume in fronto-temporo-insular regions in healthy subjects (Lane *et al.*, 2009; Thayer *et al.*, 2009; Thayer *et al.*, 2012), whereas HTD patients show a disrupted association between brain volume and interoceptive performance (Yoris *et al.*, 2018). In sum, HRV measures can be combined with HEP and neuroimaging to track the neurovisceral bases of stress in HTD.

We pursued this goal using the TSST, a test that reliably induces psychosocial stress in healthy and clinical populations (Heim *et al.*, 2000; Dickerson & Kemeny, 2004; Kudielka *et al.*, 2007), including HTD patients (Wirtz *et al.*, 2006). Specifically, the TSST achieves this by combining social and cognitive components that entail evaluative threat and outcome uncontrollability –two

factors that surpass other stressors in eliciting stress responses such as cortisol levels (Dickerson & Kemeny, 2004). Our multimodal approach encompasses measurements of a critical HRV feature (LF/HF ratio), neurophysiological (HEP), and neuroanatomical correlates (voxel-based morphometry [VBM]). Considering previous findings (Wirtz *et al.*, 2006; Lane *et al.*, 2009; Thayer *et al.*, 2012; MacKinnon *et al.*, 2013; Schulz *et al.*, 2013; Garafova *et al.*, 2014; Kleckner *et al.*, 2017; Yoris *et al.*, 2018; Mocayar Marón *et al.*, 2019), we predicted that HTD patients would exhibit significantly increased and undifferentiated HRV (LF/HF ratio) during baseline and acute mental stress induced through the TSST. In this sense, higher HRV was expected to be accompanied by increased HEP modulations, associated to allostatic/interoceptive dysregulations. Finally, we expected that HTD patients would exhibit reduced associations between HRV and the volume of key fronto-temporo-insular regions. In brief, this multimodal study aims to shed novel light on multidimensional markers of health and mental stress.

Materials and methods

Participants

The study comprised 25 HTD patients and 27 healthy controls with no history of hypertension. Power estimation analysis confirmed the robustness of our sample size (Information S1). Subjects in the HTD group were chronic outpatients of the Metabolic and Arterial Hypertension Unit of the Favaloro Foundation Hospital, diagnosed following current revised criteria (Sánchez *et al.*, 2003). Their blood pressure fell in Grade 1 (systolic blood pressure between 140–159 mmHg and/or diastolic blood pressure between 90–99 mmHg), within the hypertension classification of the 2007 European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) guidelines for the management of arterial hypertension (Mancia *et al.*, 2007). Office blood pressure readings and ambulatory blood pressure monitoring confirmed the patients' condition (Table 1). All subjects in the HTD group were under antihypertensive medication (Table S1), but intake was suspended 48 hours before the study to prevent drug-related confounds (Yoris *et al.*, 2018). None of the participants presented psychiatric symptoms, as assessed through extensive clinical interviews based on the WHO's ICD-10 diagnostic guidelines (WHO, 1992). Additionally, neither controls nor patients presented cognitive or emotional impairments (for details, see Cognitive and emotional assessment section, and Table 1), lacunar infarcts, or white (WM) or grey matter (GM) lesions (Figure S1). Both groups were matched for gender, age, education, and body

mass index (Table 1). All participants signed an informed consent in accordance with the World Medical Association Declaration of Helsinki. The study was approved by the ethics committee of the Institute of Cognitive Neurology.

Cognitive and emotional assessment

All participants were evaluated with the INECO Frontal Screening (IFS) battery (Torralva *et al.*, 2009), which is sensitive to executive dysfunction (Torralva *et al.*, 2009; Gleichgerrcht *et al.*, 2011). This test includes eight subtests: (1) motor programming (Luria series, ‘‘fist, edge, palm’’); (2) conflicting instructions (subjects must hit the table once when the administrator hits it twice, or twice when the administrator hits it only once); (3) motor inhibitory control; (4) numerical working memory (backward digit span); (5) verbal working memory (months backwards); (6) spatial working memory (modified Corsi tapping test); (7) abstraction capacity (inferring the meaning of proverbs); and (8) verbal inhibitory control (modified Hayling test). The maximum possible score on the IFS is 30 points. Furthermore, we used the Beck Depression Inventory (BDI-II) (Beck *et al.*, 1996), a 21-item scale for the assessing of emotional, behavioral, and somatic symptoms of depression. Higher BDI-II scores indicate more severe depression symptoms. Between-group comparisons assessed through one-way ANOVA indicated non-significant differences between controls and HTD patients (Table 1).

Trier Social Stress Test

Responses to stress were assessed with the TSST (Kirschbaum *et al.*, 1993; Allen *et al.*, 2017), a gold-standard test to induce acute psychosocial stress, as indexed through key biomarkers (Dickerson & Kemeny, 2004; Allen *et al.*, 2014). In light of our hypothesis, we focused on two periods of the test: the baseline period (used to determine a basal state) and the stress period (which involves three stressful tasks: speech preparation, speech, and mental arithmetic). First, in the baseline period (lasting 5 minutes), participants were asked to remain still, keep their eyes open, and avoid thinking about anything in particular. Then, subjects were given 5 minutes to prepare a speech and convince an evaluator that they were perfect candidates for a job (Speech preparation). Immediately after, participants delivered their monologue facing an unfamiliar evaluator trained to withhold any type of social engagement or positive feedback (Speech). Finally, participants completed a 5-minute serial-subtraction task (Mental arithmetic), assessed by the same evaluator. ECG and EEG signals were recorded during the whole test to assess HRV and

heart rate (see ECG data preprocessing and analysis section for details), as well as HEP modulations (see EEG data preprocessing and analysis section for details), respectively.

State-anxiety assessment during TSST

Exposure to stress generally leads to subjectively negative experiences (Allen *et al.*, 2014), and the TSST, in particular, increases anxiety (Kirschbaum *et al.*, 1993; Rohrmann *et al.*, 1999; Rimmele *et al.*, 2009). To confirm that participants, especially the HTD sample, found the TSST stressful, we compared state anxiety using the state scale of the State-Trait Anxiety Inventory (STAI) (Speilberger & Vagg, 1984) before baseline and immediately after stress period. Two-tailed paired *t*-tests showed increased anxiety across groups immediately after the stress period, evidencing that the task was actually stressful (for details, see Table 2).

ECG data preprocessing and analysis

Preprocessing of heart rate data

We measured HRV to estimate the influence of cardiodynamic differences between groups during the TSST (Castaldo *et al.*, 2015). We used the Kubios HRV program (Tarvainen *et al.*, 2014) and imported the beat-to-beat RR interval data from the ECG (on the MATLAB platform [MATLAB R2017b, The MathWorks, Natick, MA, USA]). This software analyzes HRV in both time and frequency domains. Given the short timespans, we applied an autoregressive algorithm to analyze the power spectrum (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). This algorithm generates a power spectral analysis with different frequency bands: high frequency (HF: 0.15 to 0.40 Hz), low frequency (LF: 0.04 to 0.15 Hz), and very low frequency (VLF: 0.00 to 0.04 Hz). The LF/HF ratio has been suggested as a measure of sympathovagal balance (Eckberg, 1997; Taelman *et al.*, 2009). We calculated these frequency components in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component (Pagani *et al.*, 1986; Malliani, 1991). Moreover, The Kubios HRV also yields a mean heart rate measure for each period.

HRV analysis

HRV was analyzed based on normalized units from the LF/HF ratio. To remove data unrelated to psychophysiological process and enhance the power, values above 2 SDs LF/HF ratio were

excluded (Zimmerman, 1994; Osborne & Overbay, 2004; de la Fuente *et al.*, 2019). The mean HRV (LF/HF) modulation during the TSST periods (baseline and stress) was compared between groups via a one-way ANOVA. Effect sizes were reported with partial eta squared (ηp^2). Between-group comparisons were performed on SPSS software (version 22.0, IBM Corp, Armonk, NY, USA).

Heart rate analysis

Acute stress raises heart rate, as confirmed in previous TSST studies (Buske-Kirschbaum *et al.*, 2002; Rimmele *et al.*, 2007; Yamakawa *et al.*, 2009). We assessed heart rate differences between periods for each group via two-tailed paired *t*-tests. Both groups presented a significant increase in heart rate between baseline and stress periods (see Table 3)**,** confirming that the stress period of the TSST was actually stressful.

EEG data preprocessing and analysis

EEG signal preprocessing

Data was obtained with a Biosemi Active-two 128-channel system at 1024 Hz. Preprocessing and analysis were performed using EEGLAB functions (EEGLAB 14.1.1b, University of San Diego, San Diego, CA, USA), running under MATLAB. EEG data was resampled offline at 256 Hz and band-pass filtered during recording (0.1 to 100 Hz) and off-line (0.3 to 50 Hz) to remove undesired frequency components (Yoris *et al.*, 2018). The reference was set by default to link mastoids. EEG data were segmented between -200 to 700 ms of the ECG R-wave peak. Following a widely reported EEG approach (Couto *et al.*, 2014; Couto *et al.*, 2015a; García-Cordero *et al.*, 2016; Garcia-Cordero *et al.*, 2017; Yoris *et al.*, 2017; Salamone *et al.*, 2018; Yoris *et al.*, 2018; Fittipaldi *et al.*, 2020), segments with cardiac-field artifacts and eye movement contamination (including slow and fast blinks, and vertical and horizontal eye movements) were rejected via independent component analysis (ICA). In this analysis, spatial filters are derived by producing the set of maximally temporally independent signals in the EEG data (Delorme *et al.*, 2007). The components of the data that resemble electromyogenic artifacts (broad bandwidth, peripheral distribution) were selected through visual inspection by a well-trained expert and projected out of the channel, leaving clean data only. To avoid possible biases related to the rejection criteria, the same expert was in charge of the whole visual inspection procedure, a standard for artifact

rejection in EEG research (Muthukumaraswamy, 2013). The epochs were baseline-corrected (baseline: -200 ms to 0 ms) (Szczepanski *et al.*, 2014). Finally, noisy epochs were also rejected following a visual inspection procedure (Yoris *et al.*, 2018). No significant differences were found in the number of remaining epochs between groups in each period (Information S3).

HEP analysis

The HEP was obtained by sampling EEG epochs time-locked to the R-wave. HEP modulations between TSST baseline and TSST stress were analyzed through a point-by-point Monte Carlo permutation test (5000 permutations, $P < 0.05$) (Manly, 2006), as done previously with this ERP (Couto *et al.*, 2015b; García-Cordero *et al.*, 2016; Yoris *et al.*, 2018; Salamone *et al.*, 2020) and other components (Ibanez *et al.*, 2012; Ibáñez *et al.*, 2013; Amoruso *et al.*, 2014; Melloni *et al.*, 2016). This method offers a solution to the multiple comparisons problem and does not depend on multiple comparison corrections or Gaussian distribution assumptions (Nichols & Holmes, 2002). We analyzed the HEP signal only after the 200-ms mark to avoid the influence of cardiac field artifacts (Kern *et al.*, 2013). HEP analyses were based on an extended frontal region of interest (ROI) (40 frontal electrodes: Biosemi C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 C18 C19 C20 C21 C22 C23 C24 C25 C26 C27 C28 C29 C30 C31 C32 D1 D2 D3 D4 D5 D6 D7 D8) (Figure 1b) (Pollatos & Schandry, 2004; Gray *et al.*, 2007; Yoris *et al.*, 2018). To examine the topographic distribution within the extended frontal ROI, we also assessed three separate sub-ROIs within the above set of electrodes, namely: a right frontal (Biosemi C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13), a left frontal (Biosemi C24 C25 C26 C30 C31 C32 D1 D2 D3 D4 D5 D6 D7), and a central frontal (Biosemi C14 C15 C16 C17 C18 C19 C20 C21 C22 C23 C27 C28 C29) ROI (Figure S2).

Associations between HEP and HRV

Pearson's correlation analyses ($P < 0.05$) were used to examine a possible association between HEP modulation and HRV during the TSST, in both groups. This was done by calculating the mean average of the significant window of the HEP modulation during each TSST period (baseline and stress).

MRI Measures

MRI acquisition and preprocessing

MRI acquisition and preprocessing steps are reported as recommended by the Organization for Human Brain Mapping (Nichols *et al.*, 2017; Poldrack *et al.*, 2017). Eight subjects from the original sample were excluded due to absence of imaging data, artifacts or acquisition problems. The final MRI sample consisted of 23 controls and 21 HTD patients demographically matched (see Table S2). Using a 1.5-T Phillips Intera scanner with a standard head coil (8 channels), we acquired whole-brain T1-weighted anatomical 3D spin echo volumes, parallel to the plane connecting the anterior and posterior commissures, with the following parameters: repetition time (TR) = 7489 ms; echo time (TE) = 3420 ms; flip angle = 8º; 196 slices, matrix dimension = 256 x 240; voxel size = 1 x 1 x 1 mm3; sequence duration = 7 min.

The resulting images were preprocessed on the DARTEL Toolbox, following validated procedures (Ashburner & Friston, 2000; Yoris *et al.*, 2018), and analyzed on Statistical Parametric Mapping software (SPM12, Wellcome Department of Cognitive Neurology, University of London, London, UK, available in http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) running on MATLAB. T1-weighted images in native space were first segmented using the default parameters of SPM12 (bias regularization was set to 0.001, and bias FWHM was set to 60-mm cut-off) into WM, GM, and cerebrospinal fluid (used to estimate the total intracranial volume). Then we ran the "DARTEL (create template)" module using the GM and WM segmented images—with the default parameters— to create a template from the complete data set (to increase the accuracy of inter-subject alignment) (Ashburner, 2007). Then, we used the "Normalise to MNI Space" module from DARTEL Tools to affine register the last template from the previous step into the MNI Space. This transformation was then applied to all the individual GM segmented scans to also be brought into standard space. Subsequently, all images were modulated to correct volume changes by Jacobian determinants, and avoid a bias in the intensity of an area due to its expansion during warping. Finally, data were smoothed using an 8 mm full-width-at-half-maximum isotropic Gaussian kernel to accommodate for inter-subject differences in anatomy. The size of the kernel was selected based on previous recommendations (Good *et al.*, 2001).

Association between HRV during TSST and neuroimaging measures

Regression analyses were performed to account for the relation between HRV in TSST periods (baseline and stress) and GM, using the outputs from VBM preprocessing. Given that HRV has been related to a widely distributed set of fronto-temporo-insular regions (Lane *et al.*, 2009; Thayer *et al.*, 2012), we applied a specific mask comprising these areas (anterior cingulate cortex,

orbitofrontal cortex, gyrus rectus, inferior frontal gyrus, anterior frontal middle gyrus, amygdala, basal ganglia [caudate nucleus, putamen, pallidum], insula, hippocampus, and parahippocampus) and performed a voxel-wise analysis inside them (Sedeno *et al.*, 2016; de la Fuente *et al.*, 2019; Bachli *et al.*, 2020). This mask was developed based on the regions of the Automated Anatomical Labeling (AAL) Atlas (Tzourio-Mazoyer *et al.*, 2002). Total intracranial volume was used as a covariate to discard the influence of brain-size differences. However, differences in overall brain volume between groups were assessed through one-way ANOVA. No significant differences were found ($F_{1,42}$ = 0.00, P = 0.992, $\eta p^2 < 0.01$) between patients (M = 1386.73, SD = 128.83) and controls (M = 1386.35, SD = 122.66). Statistical significance was set at $P < 0.001$ uncorrected, extent threshold = 30 voxels (Steeb *et al.*, 2018; de la Fuente *et al.*, 2019). A power estimation analysis confirmed the robustness of our results (Information S2).

Results

HRV during the TSST

Between-group comparisons of HRV during the TSST via one-way ANOVA revealed significant differences for the baseline period ($F_{1,46}$ = 10.54, P = 0.00, ηp^2 = 0.18), such that patients (M = 4.30, SD = 3.97) showed higher LF/HF ratio than controls (M = 1.58, SD = 1.27). No significant differences were found in the stress period ($F_{1,40}$ = 0.37 P = 0.54, ηp^2 = 0.009) between patients (M = 3.34, SD = 1.87) and controls (M = 3.66, SD = 1.36) (Figure 1a).

Also, to test for potential group differences in the transition between periods (specifically, the groups' adjustment to the stress period), we subtracted baseline HRV from stress HRV for each sample, thus controlling for baseline fluctuations and adjusting for normal intrasubject variability. A one-way ANOVA between both subtractions showed significant differences ($F_{1,38}$ = 6.37 P = 0.01, ηp^2 = 0.14), with lower values for patients (M = -0.60, SD = 2.99) than controls (M = 1.63, SD = 1.78) (Figure 1a).

The same results were obtained when the sample was analyzed without excluding outliers. Between-group comparisons of HRV during the TSST revealed significant differences for the baseline period ($F_{1,50}$ = 5.39, P = 0.02, ηp^2 = 0.09), such that patients (M = 4.82, SD = 5.20) showed higher LF/HF ratio than controls (M = 2.20, SD = 2.60). In the stress period, no significant differences were found ($F_{1,50}$ = 0.04, P = 0.82, ηp^2 = 0.001) between patients (M = 3.99, SD = 2.39) and controls (M = 4.18, SD = 3.66). Finally, when comparing the groups'

transition between periods, a one-way ANOVA between subtractions of HRV conditions showed significant differences ($F_{1,50}$ = 4.29, P = 0.04, ηp^2 = 0.07), with lower values for patients (M = -0.83, SD = 5.55) than controls (M = 1.97, SD = 4.18).

HEP modulation during the TSST and association with HRV

During the baseline period, significantly greater HEP modulations were observed in the patients compared to controls over the frontal ROI between 566 and 589 ms ($P < 0.05$) (Figure 1b). Conversely, the stress period yielded no significant differences between groups. These results were replicated ($P < 0.05$) over the right frontal [460 to 480 ms; 562 to 597 ms] and left frontal [570 to 593 ms] sites when each sub-ROI was separately assessed (Figure S2).

In the baseline period, a significant negative association between the HEP and HRV was observed in controls (r = -0.49, P = 0.02) and patients (r = -0.51, P = 0.05). Conversely, the stress period yielded no such correlation in either group (Controls: r = -0.16, P = 0.51; patients: r = -0.28, P = 0.46) (Figure 1c).

Association between HRV and GM volume

HRV during baseline was associated with the GM volume of the right middle orbital frontal cortex and left insula in controls, and with the left middle frontal cortex in patients. In contrast, during the stress period, controls presented a significant positive association between HRV and GM volume of right inferior and superior frontal cortices as well as the right putamen. No significant correlations were found in patients in the stress period (Figure 1d and Table 4).

Discussion

Our results evidence stress-related HRV dysregulation in HTD associated with electrophysiological and neuroimaging markers. Relative to controls, patients exhibited increased LF/HF ratio and greater HEP modulations during baseline, as well as reduced changes between baseline and stress periods, and lack of significant stress-related HRV associations with GM volume of putative frontrostriatal regions. This novel multidimensional evidence suggests that HTD patients present core markers of increased stress in non-stressful conditions and impaired neurovisceral responsiveness to acute stress. Altogether, our findings add to a promising theoretical and clinical agenda.

First, HTD patients exhibited increased LF/HF ratio during baseline, which has been associated to basal sympathetic hyperactivity and parasympathetic withdrawal (Wirtz *et al.*, 2006; Madsen *et al.*, 2008; Xhyheri *et al.*, 2012; Garafova *et al.*, 2014). This pattern is indicative of basal autonomic imbalance. More particularly, since these same modulations are core signatures of mental stress in healthy subjects (Kim *et al.*, 2018), our results may indirectly represent markers of basal stress in HTD, possibly emerged from a constant overreaction to daily life stress (Kaushik *et al.*, 2004). In this line, greater frontal HEP modulations found in patients during baseline, could be interpreted as an interoceptive hypervigilance driven by a stress overload. Indeed, cortisol levels have been related to HEP amplitudes (Schulz *et al.*, 2013). Also, the absence of significant associations between HRV and GM volume of key brain regions subserving cardiac activity strengthens the view that neurovisceral regulations are disrupted in HTD compared to controls. Indeed, controls' HRV was related with regions' GM volume underlying adaptive behavior, emotion regulation, and autonomic regulation (Lane *et al.*, 2009; Thayer *et al.*, 2012), namely: the right middle orbital frontal cortex (Thayer *et al.*, 2009) and left insula (Oppenheimer *et al.*, 1996; Ibanez *et al.*, 2010; Wei *et al.*, 2018; Ibáñez, 2019). The association between HRV and HEP in controls (MacKinnon *et al.*, 2013; Shaffer *et al.*, 2014) was replicated during baseline. However, the patients' reduced association (only marginally significant) might suggest suboptimal neurovisceral interactions. Briefly, these results indicate that, even in the absence of explicit stressors, HTD patients present multimodal signatures of altered stress-related mechanisms, suggesting a basal stress overload effect.

The above interpretation is consistent with results from the stress period. Although no HRV differences were found between groups during stress, the significantly smaller between-periods changes in LF/HF ratio observed in HTD reflects the absence of HRV adjustment to acute mental stress. The lack of endogenous variability in neurally mediated peripheral systems reinforces the view of impaired autonomic responsiveness (Madsen *et al.*, 2008; Thayer *et al.*, 2010; Shaffer *et al.*, 2014). Indeed, this pattern could be explained by the exacerbated modulations of these markers during baseline. However, other studies did report increased sympathetic activity during stress in HTD (Kaushik *et al.*, 2004; Nyklicek *et al.*, 2005; Wirtz *et al.*, 2006). While that may seem at odds with our findings, most of these studies used autonomic measures that differ from the present HRV index. Also, studies that use the LF/HF ratio tracked markedly younger samples (characterized by increased sympathetic activity) and suboptimal stress-inducing methods (Ruediger *et al.*, 2004; Garafova *et al.*, 2014). Moreover, during the stress period, the lack of

significant GM associations with HRV modulations in the HTD group compared to controls and altered HEP modulations (Gray *et al.*, 2007) supports the hypothesis of homeostatic disruptions and neurovisceral interaction disturbances in acute stress. Indeed, these dynamics are markedly different in healthy subjects. As corroborated in our study, normotensives show greater between-periods changes in LF/HF ratio, as well as right-sided frontrostriatal GM volume correlates during task-evoked ANS regulation (Lane *et al.*, 2009; Thayer *et al.*, 2009; Wei *et al.*, 2018). The absence of these markers of normal autonomic reactivity to stress (Delaney & Brodie, 2000; Castaldo *et al.*, 2015; Kim *et al.*, 2018) and healthy ANS balance (Thayer *et al.*, 2010) in HTD could explain the loss of significant differences between groups in HRV modulation and HEP amplitude during stress. Altogether, these findings further point to defective multidimensional responsiveness to acute mental stress in HTD patients.

Our findings carry theoretical and clinical implications. Basal autonomic imbalance and the lack of adjustment to acute stress in HTD could be explained through the Neurovisceral Integration Across a Continuum of Time (NIACT) model (Kemp *et al.*, 2017), a novel temporal framework which characterizes HRV as a link between psychological moments and mortality risk. Specifically, it conceptualizes how everyday moments both affect and are affected by the HRV in ways that have long-term effects on mortality risk, since it indexes the vagus nerve –which plays a critical role over allostatic systems. Consequently, basal HRV may index everyday psychophysiological resources, providing the best indication of wellbeing and future health. Conversely, task-driven activity, including psychosocial stress, would reflect autonomic responsiveness (Kemp *et al.*, 2017). In this line, increased basal LF/HF ratio in HTD suggests peripheral cardiovascular disruptions triggering allostatic load (McEwen, 1998). Our results also extend the NIACT model onto neurocognitive dimensions, by showing the HEP and GM density in fronto-temporo-insular areas constitute key markers of neurovisceral interaction. For instance, the differential HEP modulation observed between groups at baseline may reflect the abovementioned allostatic overload in HTD. This aligns with an embodied perspective suggesting that peripheral cardiovascular impairments, previously associated to interoceptive deficits (Yoris *et al.*, 2018), may disrupt allostatic-interoceptive dynamics, preventing successful allostasis. More specifically, the greater frontal HEP modulations found in patients during baseline could be interpreted as interoceptive hypervigilance driven by stress-related allostatic overload. Moreover, the pathophysiological implications of this state (Karatsoreos & McEwen, 2011) might explain the development and maintenance of hypertension (Liu *et al.*, 2017b; Mocayar Marón *et al.*, 2019)

and its detrimental impact on patients' health (Ruediger *et al.*, 2004; Thayer *et al.*, 2010). Further developments along these lines can lead to more comprehensive modelling of stress in healthy and pathological populations.

From a clinical perspective, our results highlight basal HRV monitoring as an objectively cardio-cognitive marker in risk populations. Similarly, multilevel neurovisceral indicators could afford objective assessments of the patients' ability to adaptively react to acute psychosocial stressors, complementing standard self-report evaluations (Kim *et al.*, 2018). In line with previous works (García-Cordero *et al.*, 2016; Salamone *et al.*, 2018; Yoris *et al.*, 2018; Fittipaldi *et al.*, 2020), these multimodal insights underscore the relevance of neurocognitive data for the development of applied innovations in the diagnosis, prognosis, and treatment of conditions with autonomic imbalance and increased morbidity (Thayer *et al.*, 2010; Castaldo *et al.*, 2015; Allen *et al.*, 2017; Kemp *et al.*, 2017).

Limitations and avenues for further research

The present work features some limitations and opens a new agenda for further research. First, it is based on a modest sample size. However, it is adequate according to power estimation analysis (Information S1 and S2) and similar to other works with convergent results (Wirtz *et al.*, 2006; Garafova *et al.*, 2014). Moreover, the consistency of our results across cardiodynamic, electrophysiological, and neuroimaging dimensions, with moderate to large effect sizes, further attests to their robustness. Still, non-significant results may vary with larger samples, calling for further replication. Second, our findings may have been partially influenced by the patients' medication. However, following previous reports (Yoris *et al.*, 2018), we suspended intake 48 hours before testing to minimize its potential impact. Still, valuable information could be obtained in future works by comparing basal and acute stress levels in treated and untreated patients. Another possible limitation is the restricted range of stress parameters. Although HRV and HEP are valid proxies to assess acute mental stress, future studies should include other parameters, like salivary cortisol (Kirschbaum & Hellhammer, 1994; Kudielka *et al.*, 2007) hypothalamus–pituitary–adrenal axis activity, sympathetic-adrenal-medullary activation or immune system activity measurements (Nyklicek *et al.*, 2005; Wirtz *et al.*, 2006). Moreover, the sensitivity of the LF/HF ratio to sympatho-vagal balance is debated, especially in short-term resting paced breathing (at 0.1 Hz) recordings, where LF power may be almost entirely vagally mediated (Billman, 2013; Shaffer *et al.*, 2014). Nonetheless, in our work, we assumed that the LF component mainly

reflected sympathetic activity, since the TSST involved a baseline period assessed at normal breathing and a psychosocial stress period that has been widely validated to produce sympathetic activation (Allen *et al.*, 2014; Allen *et al.*, 2017). We encourage future studies to take this issue into consideration. Finally, between-group HEP differences in the present study emerged in a short time span. However, this ERP may manifest in windows of variable extension (Gray *et al.*, 2007; Schulz *et al.*, 2015; Salamone *et al.*, 2018; Salamone *et al.*, 2020). Future studies could illuminate which subject- or task-related factors determine the duration of (stress-sensitive) HEP effects.

Conclusions

Our study revealed an autonomic imbalance in HTD patients, reflected in a potential basal stress overload effect and a lack of regulation to acute psychosocial stress, accompanied by neurophysiological and neuroanatomical alterations. Further work along these lines could better elucidate the multidimensional signatures of stress in HTD patients and normotensives, yielding new insights into heart-brain interactions.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

Study Design: AY, AI, LS.

Data collection: AY, SA, MM, FA.

Data processing and analysis: AL and LS.

Wrote manuscript: AL, AI, and AG.

All authors have participated sufficiently in the work and approve the final version of the manuscript for submission.

Data Accessibility Statement

Experimental data is available online on the Open Science Framework at: https://osf.io/ybgn5.

Abbreviations

ANS: autonomic nervous system

GM: grey matter

HEP: heart-evoked potential

HRV: heart rate variability

HTD: hypertensive disease

LF/HF: low frequency/high frequency

NIACT: Neurovisceral Integration Across a Continuum of Time model

ROI: region of interest

SPM12: Statistical Parametric Mapping software v.12

TSST: Trier Social Stress Test

VBM: voxel-based morphometry

WM: white matter

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Tables

Table 1. Demographic, cardiovascular, cognitive and emotional measures

Variables	Groups		Statistics
	Controls	HTD patients	
Demographic results			
Gender (F:M)	18:09	13:12	$\chi^2 = 0.63$, $P = 0.43$
Age	66.04 (7.29)	67.56 (9.34)	$F_{1,50} = 0.43$, $P = 0.51$, $\eta p^2 = 0.00$
Education	16.59 (2.39)	15.00 (4.54)	$F_{1,50} = 2.55$, $P = 0.12$, $\eta p^2 = 0.04$
Body mass index	25.62 (3.37)	26.89 (3.45)	$F_{1,50} = 1.78$, $P = 0.19$, $\eta p^2 = 0.03$
Handedness (R:L)	26:0	23:01	---
Clinical cardiovascular assessment			
ABPM (systolic 24hrs.)	114.47 (18.21)	127.60 (13.25)	$F_{1,37} = 6.67$, $P = 0.01$*, $\eta p^2 = 0.15$
OBP systolic	132.85	143.51	$F_{1,33} = 4.19$, $P = 0.04$*, $\eta p^2 =$

	(13.10)	(17.74)	0.11
OBP diastolic	77.68 (6.99)	82.31 (4.92)	$F_{1,31} = 4.46$, $P = 0.04$*, $\eta p^2 = 0.12$
Cognitive symptom assessment			
IFS	25.26 (2.35)	23.95 (3.68)	$F_{1,48} = 2.27$, $P = 0.13$, $\eta p^2 = 0.04$
Emotional symptom assessment			
BDI – II	11.47 (9.10)	9.38 (4.66)	$F_{1,32} = 0.58$, $P = 0.450$, $\eta p^2 = 0.18$

Results are presented as mean (*SD*). The asterisk indicates significant differences. Demographic and clinical data were assessed through ANOVAs. Gender was analyzed with Pearson's chi-squared (χ^2) test. Effects sizes were calculated through partial eta (ηp^2). ABPM: ambulatory blood pressure monitor; BDI-II: Beck Depression Inventory – II; IFS: INECO Frontal Screening; OBP: office blood pressure.

Table 2. State-anxiety during TSST

Groups	**Variables**		**Statistics**
	State-anxiety baseline	**State-anxiety stress**	
All groups	27.05 (6.31)	31.16 (8.26)	$t_{47} = -3.27$, $P = 0.002$*, $d = 0.47$
Controls	28.03 (4.53)	30.03 (6.74)	$t_{25} = -1.44$, $P = 0.162$, $d = 0.28$
HTD patients	26.86 (7.98)	32.50 (9.75)	$t_{21} = -3.21$, $P = 0.004$*, $d = 0.68$

State-anxiety was assessed through the state scale of the State-Trait Anxiety Inventory (STAI) (Speilberger & Vagg, 1984). Results are presented as mean (*SD*). The asterisk indicates significant difference. Effect sizes were calculated through Cohen's d. HTD: Hypertensive disease.

Table 3. Heart rate during TSST

Groups	Variables		Statistics
	HR baseline	**HR stress**	
Controls	65.46 (5.15)	72.73 (5.24)	$t_{24} = -7.67$, $P < 0.001$*, $d = 1.53$
HTD patients	68.73 (9.13)	84.19 (21.43)	$t_{20} = -3.28$, $P = 0.004$*, $d = 0.71$

Results are presented as mean (SD). The asterisk indicates significant differences. Effect sizes were calculated through Cohen's d. HTD: Hypertensive disease; HR: Heart rate.

Table 4. Regression between GM in fronto-temporo-insular regions and HRV during the TSST

Brain region	Peak *t*	Peak *P* (uncl)	*x*	*y*	*z*
Controls - TSST baseline					
Middle orbitofrontal R	5.53	< 0.001	37.5	57	-1.5
	5.46	< 0.001	42	51	-9
	4.63	< 0.001	42	46.5	-18
	5.27	< 0.001	-45	42	-18
Insula L	4.78	< 0.001	-42	4.5	-1.5
Controls - TSST stress					
Putamen R	5.29	< 0.001	28.5	18	6

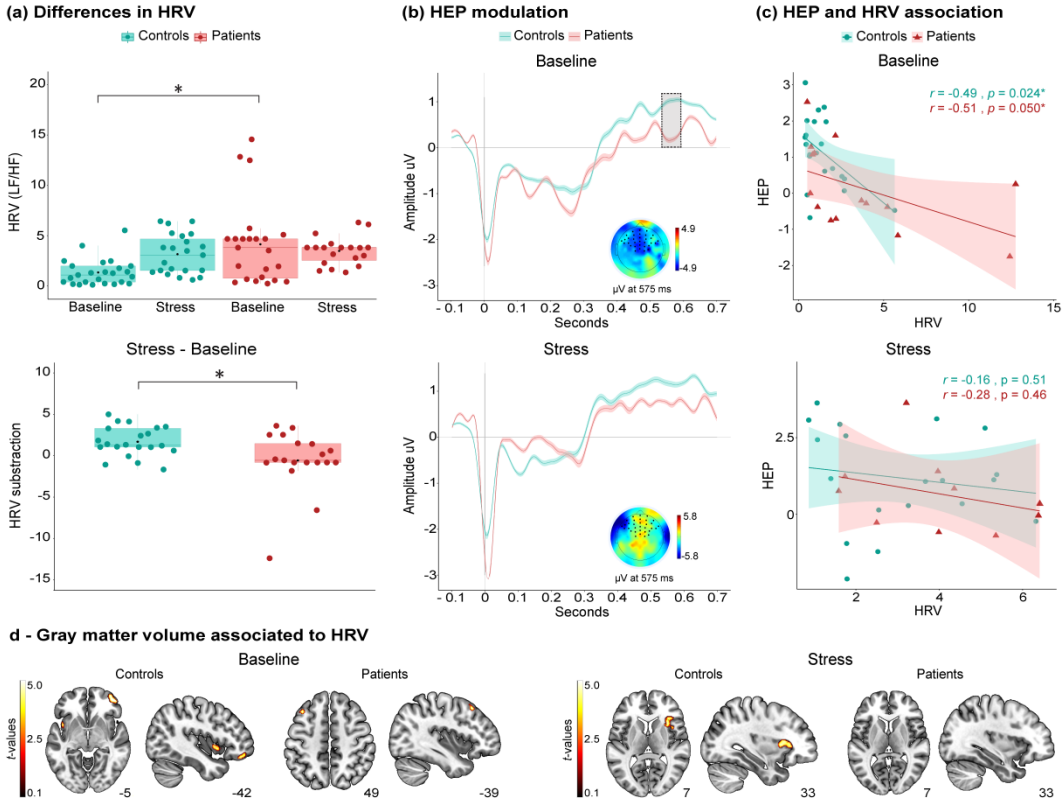
	4.56	< 0.001	34.5	25.5	10.5
	4.20	< 0.001	42	10.5	12
Inferior orbitofrontal R	4.35	< 0.001	49.5	33	-6
Superior orbitofrontal R	4.28	< 0.001	27	58.5	0
HTD patients - TSST baseline					
Middle frontal L	5.17	< 0.001	-39	24	49.5

Regressions between GM volume and HRV during TSST: No associations between GM volume and HRV during TSST stress were found in HTD patients. HTD: Hypertensive disease; R: Right; L: Left.

Figure captions

Figure 1. (**a**) **Differences in HRV during the TSST:** Baseline and stress periods (superior row), subtraction between stress and baseline (inferior row). Significant differences are indicated with an asterisk ($P < 0.05$). Individual modulations are represented inside and outside the box as points. The middle box line indicates the group's mean values. (**b**) **HEP modulation in frontal ROI during the baseline and stress periods during the TSST:** Differences between groups were calculated via Monte Carlo permutations analyses (5000 permutations, $P < 0.05$), point by point (Manly, 2006). Following previous reports, a minimum extension of five consecutive points was establish to ensure temporally robust results (García-Cordero *et al.*, 2016; Salamone *et al.*, 2018). Shadowed bars around ERP modulations indicate SEM. Grey boxes indicate significant differences. (**c**) **Association between HEP modulation and HRV during the baseline and stress periods of the TSST**: Significant correlations are indicated with an asterisk ($P < 0.05$). (**d**) **Regressions between GM volume and HRV during the baseline and stress periods of the TSST:** these analyses were conducted to identify regions in each group that were associated with the HRV during the TSST ($P < 0.001$ uncorrected, extent threshold = 30 voxels). Baseline (left),

stress (right). HEP: Heart evoked potential; HRV: Heart rate variability; HTD: Hypertensive disease; TSST: Trier Stress Social Test.



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