Psychoneuroendocrinology 102 (2019) 196–203

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

# Vagal effects of endocrine HPA axis challenges on resting autonomic activity assessed by heart rate variability measures in healthy humans

Agorastos Agorastos[a,b,*,1], Alexandra Heinig[a,1], Oliver Stiedl[c,d], Torben Hager[c], Anne Sommer[a], Jana C. Müller[a], Koen R. Schruers[e], Klaus Wiedemann[a], Cüneyt Demiralay[a]

[a] Department of Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf, D-20246, Hamburg, Germany
[b] Department of Psychiatry, Division of Neurosciences, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, GR-54124, Thessaloniki, Greece
[c] Center for Neurogenomics and Cognitive Research, VU University Amsterdam, NL-1081, HV, Amsterdam, the Netherlands
[d] Department of Health, Safety and Environment, VU University Amsterdam, NL-1081, BT, Amsterdam, the Netherlands
[e] School of Mental Health and Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, NL-6200 MD, Maastricht, the Netherlands

ARTICLE INFO

*Keywords:*
Hypothalamus-pituitary-adrenal axis (HPA axis)
Autonomic nervous system
Heart rate variability
Stress
Metyrapone
Dexamethasone
Cortisol
Endocrine challenge
Vagus

ABSTRACT

*Background:* The hypothalamic-pituitary-adrenal axis (HPA axis) and the autonomic nervous system (ANS) are considered to play the most crucial role in the pathophysiology of stress responsiveness and are increasingly studied together. However, only few studies have simultaneously assessed HPA axis and ANS activity to investigate their direct interaction in pathophysiology, while no study so far has assessed the dynamic interplay between the two systems in healthy subjects through endocrine challenges.
*Methods:* The present study assessed the direct effects of overnight pharmacoendocrine HPA axis challenges with dexamethasone (suppression) and metyrapone (stimulation) on ANS activity at rest as determined by linear and nonlinear measures of heart rate variability (HRV) in 39 young healthy individuals.
*Results:* Findings indicated significant effects of metyrapone, but not dexamethasone on autonomic activity at rest based on HRV measures. HRV after metyrapone was overall significantly reduced in comparison to baseline or post-dexamethasone conditions, while the combined metyrapone-related reduction of HRV measures RMSSD, NN50(%) and HF(%) with concomitant increase of the unifractal scaling coefficient $\alpha_{fast}$ value jointly indicated a specifically diminished vagal activity.
*Conclusions:* We provide first data that HPA axis stimulation (metyrapone) is associated with reduced vagal tone, while HPA axis suppression (dexamethasone) has no effect on autonomic modulation of heart function. Our results support a vital role of the parasympathetic nervous system in the interplay between ANS and HPA axis and, thus, in the modulation of stress-related cardiovascular responsiveness and the susceptibility to stress-related disorders.

## 1. Introduction

Stress is defined as the state of threatened homeodynamic balance [non-equilibrium state as opposed to homeostasis; (Ikegami and Suzuki, 2008)] of an organism (Chrousos, 2009; Ikegami and Suzuki, 2008; Koolhaas et al., 2011). Excessive stress exposure may oversensitize neuroendocrine responses to stress, leading to an altered dynamic state associated with decreased adaptability and profound effects on physiological development leading to chronic physical and mental morbidity (Chrousos, 2009; de Kloet et al., 2005; Koolhaas et al., 2011; Lupien et al., 2009; McEwen, 1998). The human 'stress' system constitutes the major dynamic regulatory domain of the organism as it orchestrates a plethora of physiological reactions to acute or chronic challenges.

The human 'stress' system includes central and peripheral components. The highly interconnected central components of the stress system are mainly located in the hypothalamus and the brainstem but are driven by inputs mainly from the prefrontal cortex and the amygdala besides the direct baroreflex-mediated feedback regulation through brainstem circuits via the nucleus tractus solitarius. The central response facilitates neural pathways enhancing adapting functions such as arousal, vigilance and focused attention, while inhibiting adaptive functions irrelevant in this emotional state such as eating, growth and reproduction. The peripheral components of the stress system include

* Corresponding author at: Dept. of Psychiatry and Psychotherapy, University Medical Center, Hamburg-Eppendorf, Martini Str. 52, D-20246, Hamburg, Germany.
*E-mail address:* aagorastos@uke.uni-hamburg.de (A. Agorastos).
[1] Equal contribution.

https://doi.org/10.1016/j.psyneuen.2018.12.017
Received 24 September 2018; Received in revised form 26 November 2018; Accepted 12 December 2018

the hypothalamic-pituitary-adrenal (HPA) axis and the limbs of the autonomic nervous system (ANS), i.e. the sympathetic (SNS) and sympatho-adrenomedullary (SAM) system and the parasympathetic system (PNS). The principal peripheral effector molecules are the HPA axis-regulated glucocorticoids (GCs), involved also in the negative feedback mechanism centrally inhibiting corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) secretion, and the SAM-regulated catecholamines nor-epinephrine and epinephrine (Chrousos, 2009; Elenkov and Chrousos, 2006; Tsigos and Chrousos, 2002; Ulrich-Lai and Herman, 2009). The HPA axis and the ANS are considered to play the most crucial role in stress-related maladaptive consequences leading to pathophysiology (Chrousos, 2009; de Kloet et al., 2005; Stratakis and Chrousos, 1995).

The HPA axis and ANS are increasingly studied together (Licht et al., 2010), as their activity normally shows a certain degree of analogy and complementarity at several neuroendocrine levels. Thereby, the physiological functions of the HPA axis depend at least in part on the ANS (Thayer and Sternberg, 2006), while on the other hand HPA axis-related GC signaling may have an important role in the regulation of ANS activity (Raison and Miller, 2003). However, only relatively few studies have simultaneously assessed HPA axis and ANS activity to investigate their direct association (Adlan et al., 2018; Wiedemann et al., 2001), while most of these studies are conducted in patients (Casement et al., 2018) or through subjective stress exposure paradigms (Ali et al., 2017; Pulopulos et al., 2018). Until now, only very few studies have assessed the direct physiological dynamic interplay between the two systems in healthy subjects through pharmacological challenges. Nonell et al. (2005), for example, report a shift of the autonomic response to an orthostatic challenge towards parasympathetic activity by prolonged but not acute hydrocortisone administration. However, the effects on unchallenged autonomic function are essentially unclear.

Thus, the main objective of our study was to assess the effects of HPA axis manipulation (i.e. stimulation and suppression of the HPA axis) on baseline ANS activity in healthy individuals. We therefore investigated autonomic activity measured by heart rate variability (HRV) at rest to overnight pharmacoendocrine HPA axis challenges with metyrapone (stimulation) and dexamethasone (suppression) in a group of healthy subjects. Metyrapone (MET) crosses the blood-brain barrier and reduces, not only at the adrenal glands but also within the brain, cortisol levels by blocking the enzymatic conversion of 11-deoxycortisol to cortisol by CYP11B1 (11-beta-hydroxylase, P-450c11), the last step in the synthesis of cortisol. This leads to a rapid fall of cortisol and an accumulation of 11-deoxycortisol, which does not inhibit ACTH secretion. This results in decreased cortisol-mediated negative feedback at hypothalamic and pituitary levels, which increases CRH and ACTH secretion. Dexamethasone (DEX) is an exogenous steroid that binds mainly to glucocorticoid receptors in the anterior pituitary gland. This results in regulatory modulation through negative feedback and suppression of ACTH and consequently lowers cortisol secretion (Cole et al., 2000). We hypothesized that stimulation of the HPA axis would be associated with reduced, while suppression with enhanced HRV.

## 2. Materials and methods

### 2.1. Study participants and inclusion criteria

The study was approved by the Ethics Review Committee (ERC) of the Hamburg Medical Board and conducted at the Department of Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf, Hamburg, Germany (file Nr. PV4161). Healthy subjects between 18–65 years of age were recruited by printed/electronical public advertisement and invited to an initial appointment in our department. After full oral and written explanation of the purpose and procedures of the investigation, written informed consent was obtained from each subject before initiating the screening procedure. Screening included a thorough physical and neurological examination, routine blood laboratory tests, urine toxicology screen, electrocardiogram (ECG) recording at rest and a structured face-to-face interview.

Exclusion criteria included: presence or history of any physical (e.g., history of any chronic or acute inflammatory, metabolic, neurological or immune system-associated medical conditions) and Axes I and II mental co-morbidities, body mass index (BMI) values beyond 18–30 kg/m$^2$, frequent usage of any either illicit or prescribed drugs or over the counter medications, current use of any psychiatric medication for at least 8 weeks, drinking of more than 100 g of alcohol per week, current adverse life events, night shifts or transcontinental flights across more than four time zones during the past four weeks, abnormal physical and neurological examinations, basic blood laboratory test values deviating from the normal range (incl. thyroid function tests, transaminases, electrolytes, $CO_2$ anion gap, fasting glucose, basic blood and coagulation tests, blood lipids, haemoglobin A1c, C-reactive protein, creatinine, folic acid, vitamin B12), positive urine toxicology screen, actual menstruation, pregnancy, nursing, or not using a reliable method of birth control, any contraindication for dexamethasone or metyrapone and pathological initial ECG. Hypothyroidism in the euthyroid state through hormonal substitution, as well as hypertension in normotensive state through antihypertensive medication, did not serve as exclusion criteria. Current or lifetime psychiatric disorders were excluded using the Structured Clinical Interview for the DSM-IV, axes I and II, assessed by a trained physician, as well as through a self-rating by the Beck's Depression Inventory (BDI-II, cut-off of 9 points). All other exclusion criteria were assessed in a clinical interview setting through study questionnaires. Study completers received a reimbursement of 50 €.

### 2.2. Study procedures

Volunteers who met study inclusion and exclusion criteria were scheduled for study initiation within 1 week of final laboratory results. Additional physical examination in the first morning before study initiation also provided assurance that no subject had any acute clinical manifestations or febrile body temperature. The study assessed autonomic nervous system activity through various HRV measures at the same time on three continuous days: Day 1 (baseline), Day 2 (post-metyrapone) and Day 3 (post-dexamethasone). All subjects were encouraged to maintain a regular sleep time starting at around 11.00 p.m. for all three nights before HRV assessment, with wake-up at 7.00 a.m. and avoidance of physical strain (e.g. physical exercise, sexual activity, etc.) on all three mornings during the study. Furthermore, participants were encouraged to use public transportation or private motor vehicles (i.e. no bicycles or walking > 500 m to exclude to potential effects of elevated physical activity on HR measures) for reaching the study facility (at 8.30 a.m.) and to avoid any intake of food or beverages (water was allowed) until completion of the assessment (10.30 a.m.). Adverse side effects were assessed through a German version of the UKU side effects rating scale (Lingjaerde et al., 1987).

### 2.3. Endocrine challenges

#### 2.3.1. Overnight metyrapone stimulation test (MST)

The MST test is considered to be a simple and sensitive alternative test to evaluate the ACTH reserve and it is useful to evaluate the response of the HPA axis (Avgerinos et al., 1996; Fiad et al., 1994). Subjects received 1 g of MET (Metopiron®, Novartis, Arnhem, Netherlands) orally at 11.00 p.m. on Day 1, to assess its effects on HRV the next morning (Day 2, approx. 9 h after metyrapone intake).

#### 2.3.2. Overnight low-dose dexamethasone suppression test (DST)

The low-dose DST is one of the commonly used tests to assess HPA axis reactivity by measuring the change in peripheral cortisol levels in response to externally administered DEX (Gwirtsman et al., 1982). Subjects received 1 mg of DEX (Fortecortin®, Merck, Darmstadt, Germany) orally at 11.00 p.m. on Day 2, to assess its effects on HRV the

next morning (Day 3, approx. 9 h after DEX intake).

The temporal order of the two endocrine challenges was chosen to avoid any interference between the two interventions, as half-life times significantly differ between metyrapone (approx. 2 h) and dexamethasone (35–54 h). Subjects were blinded with respect to the specific order of endocrine challenges.

### 2.4. Assessment of heart rate variability

#### 2.4.1. ECG recordings

After an initial blood draw (8.45 a.m. of each day), all subjects were given 15 min in sitting and 15 min in supine position in a single bedded room before the ECG recording was initiated. The ECG holter recording was initiated at 9.15 a.m. of each day. All subjects stayed in bed for 75 min with at least 60-min continuous ECG recording. The 60-min ECG recording allowed to generate a recording interval both long enough for nonlinear analysis based on long-lasting heartbeat interval correlations (see Meyer and Stiedl, 2003) without transient data loss. During this time, there was no presence of study personnel in the study room (subjects were monitored through a one-way mirror window and a room-installed microphone), while relative silence (no radio, electronic media, or disturbing conversation outside the study room) was maintained. ECG recordings were obtained throughout using a 5-lead holter recording system (Schiller medilog® AR12, Schiller Medizintechnik GmbH, Ottobrunn, Germany). Data were recorded at 4096 Hz sampling rate with 16-bit resolution and stored digitally on the recorder. ECG recording was performed by specially trained study staff.

#### 2.4.2. ECG editing

ECG data were exported as text files with time and amplitude information. These were imported into LabChart (v. 7.1, ADInstruments, Spechbach, Germany) to calculate the tachograms, while the import into LabChart allowed for manual data editing, i.e., removal of artifacts and insertion of unrecognized beats. ECG data editing was a laborious process supervised by an experienced scientist (Dr. Oliver Stiedl). All data pre-processing followed the recommendations of the HRV Task Force (Camm et al., 1996), as well as recently revised guidelines (Laborde et al., 2017) before any analysis was performed. Subsequent data analysis occurred on the basis of the edited LabChart data.

#### 2.4.3. HRV analyses

The assessed HRV variables have been selected according to the guidelines for short-term recordings of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Camm et al., 1996), which are also supported by later studies (Jarrin et al., 2012; Meyer and Stiedl, 2003). We used the percentage (%) of the total power of each frequency band to compare the fractional energy (Camm et al., 1996) rather than the absolute energy.

a) Linear analyses: The HR patterns obtained from LabChart were analyzed using linear time domain and frequency domain using the LabChart software extension HRV. Instantaneous HR was calculated on the basis of the RR interval of the ECG signal. HRV in the time domain was calculated by taking the standard deviation of the N-N intervals (SDNN), by calculating the root mean square of subsequent N-N interval differences (RMSSD) and, by the percentage of adjacent NN intervals that differ in length by > 50 ms [NN50(%)]. HRV in the frequency domain was calculated by analysis of several frequency components (low frequency 0.04-0.15 Hz [LF] and high frequency 0.15-0.4 Hz [HF]) (Camm et al., 1996).
b) Nonlinear analyses: Unifractal nonlinear analysis of HR dynamics was performed using the detrended fluctuation analysis (DFA) (Peng et al., 1995) using customized MATLAB (MathWorks, Natick, MA, USA) scripts as described in prior studies (Meyer and Stiedl, 2003; Peng et al., 1995). DFA is derived from the random walk theory, and uses scaling coefficients (α) as measure of the correlation of heartbeats in its temporal sequence, thereby indicating ANS dysregulation. A scaling coefficient $\alpha = 1.5$ indicates 'Brownian noise' in which there only is short-term correlation, i.e., one heartbeat interval is correlated with the previous interval only. This is observed when the tonic parasympathetic outflow to the heart is blocked by atropine treatment (Meyer, 2002; Yamamoto et al., 1995) or as a consequence of heart transplantation (Meyer, 2002). A value of $\alpha = 1.0$ indicates 'pink noise' with persistent long-range correlation of successive heartbeat intervals, as observed in physiological HR dynamics of mammalian species including mice and man. A scaling coefficient of $\alpha = 0.5$ reflects 'white noise' and indicates the lack of any correlation between heartbeat intervals (randomness). The straight-line relationship is separated by a breakpoint indicating different slopes, $\alpha_{fast}$ and $\alpha_{slow}$, which are interpreted to reflect the coefficients of distinct ranges, short-range and long-range scaling, respectively (Meyer and Stiedl, 2003). Pathological states are characterized by a breakdown of long-range correlations, i.e., α strongly deviating from 1.0.

### 2.5. Laboratory assays

We determined cortisol (CORT, ng/ml), desoxy-cortisol (D-CORT, ng/ml) and adrenocorticotropic hormone (ACTH, pg/ml) plasma levels using commercially available immunoradiometric assays and radioimmunoassays (BRAHMS, Berlin, Germany; Nichols Institute, San Juan Capistrano, CA, USA; ICN Biomedicals, Carson, CA, USA). Intra- and inter-assay coefficients of variation were below 8%.

### 2.6. Statistical analyses

Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. Because of several parameters showing skewed distribution, all parameters were ln-transformed for further parametric analysis. All data are given in mean values (SEM) adjusted for age, sex, waist-hip-ratio and smoking. For presentation purposes, Table 1 and Tab. S1 include sample means in terms of geometric means on the original scale through back-transformation through exponentiation of ln-transformed data. Differences between treatment conditions (Day 1, Day 2: Post-MET, Day 3: Post-DEX; time effect) were tested for significance by one-way repeated measures analysis of variance (rmANOVA) for correlated samples controlling for age, sex, waist-hip-ratio and smoking. Pairwise comparisons between the three days were conducted by the Bonferroni post-hoc test. An error probability of $p < 0.05$ was accepted as statistically significant. Effect size is reported as partial eta squared ($_p\eta^2 = 0.01$: small effect size, $_p\eta^2 = 0.06$: medium effect size, $_p\eta^2 = 0.14$: large effect size). To correct for potentially inflated type I error because of multiple comparisons we used the false discovery rate (FDR) approach (Benjamini and Hochberg, 1995), as in our previous studies (Agorastos et al., 2013, 2014, 2015, 2016). Following a previously reported procedure (Verhoeven et al., 2005) *p*-values were corrected by the minimum positive FDR with a threshold set at 5%. Statistical analyses were conducted using the Statistical Package for Social Sciences Version 20 (SPSS, Chicago, IL).

## 3. Results

72 healthy subjects were screened. 43 participants were found eligible for participation in the study. Of those, 2 declined participation at the time of the scheduling phone call, 1 did not attend at scheduled appointment and 1 dropped out due to mild gastrointestinal side effects on Day 1 by metyrapone. We collected data from 39 healthy Caucasian study completers, found eligible for participation in the study. The final sample included 12 males (30.8%), 7 smokers (17.9%) and had a mean age of 35.7 ± 13.6 (19–65) years and a mean waist-hip-ratio of

**Table 1**
Adjusted geometric means of linear and nonlinear HRV measures across the three conditions and time effects of endocrine challenges.

| Domain | Measures | Adjusted geometric means | | | rmANOVA (time effect) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 1 Baseline | Day 2 Post-MET | Day 3 Post-DEX | Wilks 'λ | *F* | *p* | $_{p}\eta^2$ |
| Time domain | HR (bpm) | 56.94 (1.22) | 58.32 (1.02) | 58.09 (1.02) | .872 | 1.981 | .157 | .128$^{+}$ |
| | RMSSD (ms) | 62.36 (1.07) | 55.20 (1.07) | 59.03 (1.08) | .763 | 4.188 | **.026** | **.237**$^{++}$ |
| | SDNN (ms) | 98.00 (1.05) | 94.82 (1.05) | 93.13 (1.05) | .934 | .946 | .401 | .066$^{+}$ |
| | NN50 (%) | 29.05 (1.08) | 24.56 (1.10) | 24.00 (1.12) | .764 | 4.173 | **.026** | **.236**$^{++}$ |
| Frequency Domain | HF (%) | 16.20 (1.10) | 13.67 (1.10) | 17.74 (1.11) | .594 | 9.208 | **.001** | **.406**$^{++}$ |
| | LF (%) | 20.61 (1.08) | 18.99 (1.08) | 21.24 (1.09) | .909 | 1.349 | .276 | .091$^{+}$ |
| Non-linear | $\alpha_{fast}$ | .98 (1.03) | 1.04 (1.03) | .97 (1.04) | .675 | 6.496 | **.005** | **.325**$^{++}$ |
| | $\alpha_{slow}$ | .92 (1.02) | .91 (1.02) | .89 (1.02) | .903 | 1.453 | .252 | .097$^{+}$ |

Values are presented as geometric mean values (SEM) adjusted for age, sex, waist-hip-ratio and smoking. HR: heart rate; SDNN: standard deviation of the N-N intervals; RMSSD: root mean square of subsequent N-N interval differences; NN50(%): percentage of the number of pairs of adjacent NN intervals that differ in length by > 50 ms; LF: low frequency 0.04-0.15 Hz; HF: high frequency 0.15-0.4 Hz; LF (%) and HF (%): percentage of each frequency component from the total power; α: scaling coefficient alpha of nonlinear analysis. Differences between treatment conditions (i.e. Day 1: baseline; Day 2: post-dexamethasone; Day 3: post-metyrapone; time effect) were tested for significance by one-way repeated measures analysis of variance (rmANOVA) for correlated samples controlling for age, sex, waist-hip-ratio and smoking. *p*-values denoting statistically significant differences ($p < .05$) are shown in bold. FDR analysis revealed no potential type I errors. $^{+}$moderate effect size; $^{++}$large effect size.

**Table 2**
Adjusted geometric means of endocrine measures across the three conditions and time effects of endocrine challenges.

| Measures | Adjusted geometric means | | | rmANOVA (time effect) | | | |
|---|---|---|---|---|---|---|---|
| | D1 Baseline | D2 Post-MET | D3 Post-DEX | Wilks 'λ | *F* | *p* | $_{p}\eta^2$ |
| Desoxy-cortisol (ng/ml) | 2.77 (1.63) | 35.66 (1.09) | 1.99 (2.46) | .015 | 885.494 | < **.001** | **.985**$^{++}$ |
| Cortisol (ng/ml) | 280.90 (1.05) | 291.78 (1.05) | 23.41 (1.05) | .009 | 1725.158 | < **.001** | **.991**$^{++}$ |
| ACTH (pg/ml) | 29.11 (1.08) | 98.99 (1.19) | 11.35 (1.08) | .159 | 60.860 | < **.001** | **.841**$^{++}$ |

Values are presented as geometric mean values (SEM) adjusted for age, sex, waist-hip-ratio and smoking. Differences between treatment conditions (i.e. Day 1: baseline; Day 2: post-metyrapone, Post-MET; Day 3: post-dexamethasone, Post-DEX; time effect) were tested for significance by one-way repeated measures analysis of variance (rmANOVA) for correlated samples controlling for age, sex, waist-hip-ratio and smoking. ACTH: Adrenocorticotropic hormone (pg/ml). *p*-values indicating statistically significant differences ($p < .001$) are shown in bold. FDR analysis revealed no potential type I errors.
$^{+}$moderate effect size; $^{++}$large effect size.

0.81 ± 0.07 (0.69 - 0.94). None of the subjects of the final sample was receiving additional medication (i.e. antihypertensive medication or thyroid hormone substitution). Side effects as per UKU ratings did not differ significantly between days indicating no significant adverse effects (data not shown). Endocrine analyses of CORT, D-CORT and ACTH indicated a highly significant effect of time (i.e. treatment condition) in all three measures with very large effect sizes, which confirmed the expected effects of each treatment condition (CORT: Wilks $\lambda = .009$; $p < .001$, $_{p}\eta^2 = 0.991$; D-CORT: Wilks $\lambda = .015$; $p < .001$, $_{p}\eta^2 = 0.985$; ACTH: Wilks $\lambda = .159$; $p < .001$, $_{p}\eta^2 = 0.841$, cf. Table 2 and Fig. 2).

The results of rmANOVAs indicated a significant effect of time (i.e. treatment condition) on several HRV variables including RMSSD, NN50(%), HF(%) and $\alpha_{fast}$ across the three conditions with very large effect sizes (*cf.* Table 1). Post-hoc pairwise comparison revealed significant and highly significant differences between either Day 1 / Day 2 or Day 2 / Day 3. Day 2 values had significantly lower RMSSD, NN50(%), HF(%) and higher $\alpha_{fast}$ values than Day 1, while higher $\alpha_{fast}$ and lower HF(%) values than Day 3 (cf. Fig. 1). Day 1 and Day 3 values showed only a significant difference with respect to NN50(%) (Day 1 vs. Day 3). There were no statistically significant differences with respect to NN50(%), SDNN, LF(%) and $\alpha_{slow}$ between the three experimental conditions.

FDR analysis indicated no potential type I errors. There was no statistically significant correlation between HRV measures and endocrine levels whatsoever (data not shown).

## 4. Discussion

This study assessed the effects of pharmacoendocrine HPA axis manipulation through dexamethasone and metyrapone challenges (i.e. suppression and stimulation of the HPA axis, respectively) on baseline autonomic activity through assessment of HRV measures in healthy individuals. Therefore, commonly used linear and nonlinear (unifractal) methods for analysis of HRV and its dynamics were combined to examine autonomic effects at rest by the two pharmacological interventions. To the best of our knowledge, this is the first study investigating the direct interplay between the HPA axis and the resting ANS activity in healthy subjects through objective endocrine challenges and also the only study so far assessing effects of metyrapone on HRV.

The main findings of this study include i) significant differences of HRV variables with lower values of RMSSD, NN50(%), HF(%) and higher values of $\alpha_{fast}$ after metyrapone in comparison to baseline and post-dexamethasone conditions, ii) neglectable HRV differences between baseline and post-dexamethasone conditions and iii) no statistically significant differences with respect to NN50(%), SDNN, LF(%) and $\alpha_{slow}$ between the three conditions. Taken together, these findings indicate significant effects of HPA axis stimulation by metyrapone, but not suppression by dexamethasone on autonomic activity at rest as measured by HRV. HPA axis stimulation with metyrapone was associated with overall significantly reduced HRV. In particular, given that increased $\alpha_{fast}$ values reflect reduced PNS activity (Meyer, 2002; Meyer and Stiedl, 2003), the metyrapone-related combined reduction of RMSSD, NN50(%), HF(%) with an increase of $\alpha_{fast}$ values and no difference in NN50(%), SDNN, LF(%) values jointly reflects a diminished tonic parasympathetic activity (Lombardi and Stein, 2011; Meyer,

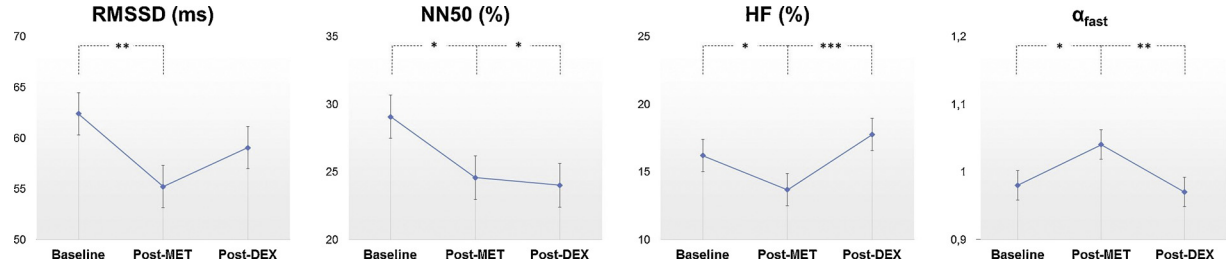


**Fig. 1.** Effects of metyrapone (MET) and dexamethasone (DEX) on HRV measures.
Pairwise comparisons between the three experimental conditions (i.e. Day 1: baseline; Day 2: post-metyrapone, Post-MET; Day 3: post-dexamethasone, Post-DEX) were conducted by the Bonferroni post-hoc test controlling for age, sex, waist-hip-ratio and smoking. Values are presented as geometric mean values ( ± SEM) adjusted for age, sex, waist-hip-ratio and smoking. RMSSD: root-mean-square of subsequent NN interval differences; NN50(%): percentage of the number of pairs of adjacent NN intervals differing in length by > 50 ms; HF (%): percentage of the high frequency (0.15-0.4 Hz) power from the total power; α: scaling coefficient alpha of nonlinear unifractal analysis. FDR analysis revealed no potential type I errors.
* $.01 \leq p < .05$, ** $.001 \leq p < .01$, ***$p < .001$.

2002), in comparison to baseline or post-dexamethasone measures at rest.

HRV results from heart rate (HR) oscillations within its physiological range (beat-to-beat variability), controlled by parasympathetic and sympathetic modulation of intrinsic cardiac pacemakers (Akselrod et al., 1981) and constitutes the best-established, non-invasive method of analysis of autonomic activity (Camm et al., 1996; Reyes del Paso et al., 2013). Heart activity under resting conditions experiences a constant tonic inhibitory control by PNS and dominance over SNS influence (Jose and Collison, 1970; Schmidt et al., 2000), as SNS cardiac influence is too slow and long-lasting to produce rapid beat-to-beat changes (Baumert et al., 1995; Kleiger et al., 1992; Meyer and Stiedl, 2003). Reduced HRV is considered a valid marker of heart disease (Thayer et al., 2012; Thayer and Sternberg, 2006), while the PNS is particularly implicated in the pathophysiology of cardiovascular diseases and other comorbidities and associated with decreased dynamic flexibility, loss of complexity and increased overall vulnerability (Thayer and Sternberg, 2006).

Since tonic parasympathetic activity underlies long-range correlation of heartbeat interval fluctuations in the healthy state (Meyer, 2002), we also included nonlinear (unifractal) HRV analysis as an important and highly sensitive readout to particularly assess vagal activity changes after challenge. Nonlinear analysis is a physiologically relevant and statistically appropriate method when analysing physiological data, because two major properties, non-stationarity (i.e. drift-like fluctuations of heartbeat intervals) and interdependence (i.e. temporal long-range correlation of heartbeat intervals), formally confound the use of linear analyses for HR data (Meyer and Stiedl, 2003). Nonlinear analysis thus provides a very sensitive measure that improves HRV assessment and is highly predictive of cardiac dysfunction attributed to autonomic dysregulation in the absence of cardiac disease (Baumert et al., 2004; Meyer and Stiedl, 2003). Unfortunately, to date, this method is still only applied in a relatively limited number of related publications on human data (Agorastos et al., 2013; Aubert et al., 2009) due to its complexity and availability of only short ECG recording intervals (commonly 5 min).

This study showed that HPA axis stimulation with metyrapone was associated with overall significantly reduced HRV due to diminished parasympathetic activity, while HPA axis suppression with dexamethasone had no effect on autonomic activity. The mechanistic understanding of ANS and HPA axis coupling could be of major importance for the understanding of the pathophysiology of stress-related disorders. Accordingly, a progressive divergence of the HPA axis and the ANS activity following stress, has been proposed as a vital pathophysiological trajectory leading to the long-term impact of excessive stress on the stress system and the chronic preservation of symptoms (Pervanidou, 2008).

HPA axis and ANS are interrelated components of an internal neural regulation system (central autonomic network, CAN) (Benarroch, 1993; Thayer and Lane, 2000). The CAN integrates high-order autonomic control associated with cognitive perception, while mediating emotional responses through hypothalamic-brainstem pathways to

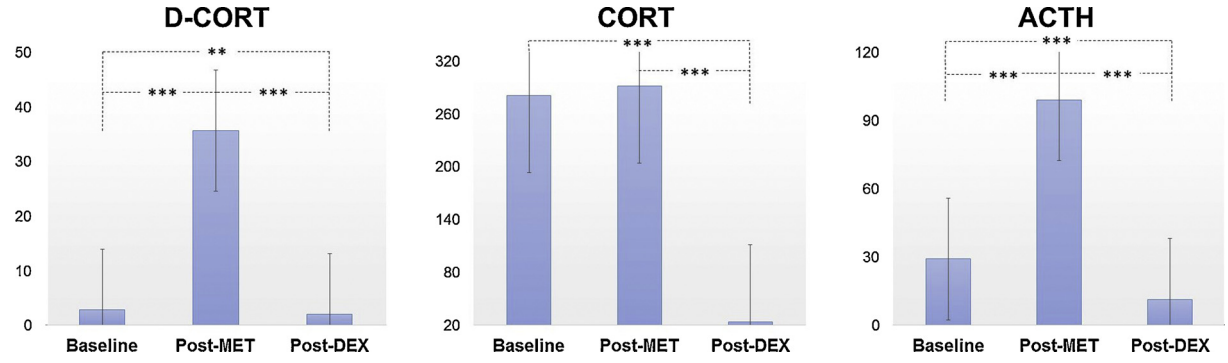


**Fig. 2.** Effects of dexamethasone (DEX) and metyrapone (MET) on endocrine measures approximately 9 h after administration.
MET and DEX were administrated at approximately 11.00 p.m. of day 1 and day 2 respectively. Endocrine measurements were conducted in plasma acquired through blood draw at approximately 08.45 a.m. of the following day (day 2 and day 3 respectively), 30 min before initiation of ECG recording. The DEX-induced D-CORT increase was indicated (left panel), the MET-induced CORT suppression was confirmed (middle panel) and the DEX-induced ACTH increase was verified. Pairwise comparisons between the three conditions (i.e. Day 1: baseline; Day 2: post-metyrapone, Post-MET; Day 3: post-dexamathasone, Post-DEX) were conducted using the Bonferroni post-hoc test to control for age, sex, waist-hip-ratio and smoking. Values are presented as geometric mean values (SEM) adjusted for age, sex, waist-hip-ratio and smoking. D-CORT: Desoxy-cortisol (ng/ml); CORT: cortisol (ng/ml); ACTH: Adrenocorticotropic hormone (pg/ml). FDR analysis revealed no potential type I errors.
*$.01 \leq p < .05$, **$.001 \leq p < .01$, ***$p < .001$.

preganglionic autonomic neurons (Thayer and Lane, 2000). For example, amygdala and brain stem neurons of the nucleus of the solitary tract are critical for both vagal activity and regulation of HPA stress responses, as parasympathetic NTS neurons send direct projections to hypophysiotropic CRH neurons in the amygdala and herewith also modulate HPA activity and its feedback control (Herman, 2017). Dysregulation of the CAN (Davis and Natelson, 1993; Loewy and Spyer, 1990; Saper, 2004) may thus affect downstream autonomic core centers and alter both peripheral ANS and HPA axis activity and responsivity (Davis and Natelson, 1993; Stiedl et al., 2010; Thayer and Lane, 2009). HRV reflects the activity of the CAN (Stiedl et al., 2010; Thayer et al., 2012) and represents a marker for regulated physical and emotional responding capacity (Thayer and Lane, 2000).

Although the precise brain processes associated with CAN and stress system dysregulation remain unclear, a central role of the hypothalamic regulatory level could be presumed. For example, CRH receptors are favorably positioned in the CNS to modulate the sympathetic and parasympathetic branches of the cardiac autonomic nervous system according to their distribution (Oliveira et al., 2015; Tran et al., 2014), including core PNS output areas (Stiedl et al., 2005). CRH has been suggested to inhibit vagal or activate sympathetic outflow at least in part via the CRH subtype 1 ($CRH_1$) receptor (Arlt et al., 2003; Nijsen et al., 2000a, b), while $CRH_1$ receptor antagonists increase cardiac vagal and decrease sympathetic activity in rats (Wood and Woods, 2007). Central CRH may thus lead to altered dynamical properties with a significant loss of intrinsic structural complexity of cardiac control due to central neuroautonomic hyperexcitation, i.e., enhanced sympatho-vagal antagonism as indicated in mice (Meyer and Stiedl, 2006; Stiedl et al., 2005). On the other hand, CRH released in the bed nucleus of the stria terminalis (BNST) during stress, contributes to cardiac stress responses, particularly by activating vagal outflow in rats (Nijsen et al., 2001). However, $CRH_1$ receptor deletion in mice has been shown to not affect heart rate adjustment and behavioural responses to acute fearful stimuli despite absent HPA axis activation (Tovote et al., 2005), which favours a central role of the suprahypothalamic rather than the hypothalamic circuitry.

Evidence suggests that the medial prefrontal cortex (mPFC) and anterior cingulate (ACC) modulate amygdala activity and that HRV reflects the strength of mPFC-amygdala interaction (Goldstein, 2012). mPFC hypofunction underlies the deficient rational control of emotional responses resulting in exaggerated amygdala activity (Falconer et al., 2008). Higher HRV was associated with stronger resting state functional connectivity (rsFC) between the amygdala and the mPFC (Sakaki et al., 2016). Interestingly, rsFC between mPFC and amydgala is majorly regulated by stress-related neuroendocrine levels (Kiem et al., 2013; Quaedflieg et al., 2015), with HPA axis stimulation (i.e. higher cortisol levels) being associated with stronger negative rsFC between these areas at rest (Veer et al., 2012). Given this, MET may lead to reduced rsFC between mPFC and amygdala (i.e., the inhibitory control of mPFC on the amygdala) and thus reduced HRV, as shown in our study (Thayer, 2006). This inhibitory control appears to be largely vagally mediated (Smeets, 2010; Thayer and Sternberg, 2006; Weber et al., 2010). This conclusion is consistent with the neurovisceral integration model, which underlines the importance of the PNS in providing negative feedback on sympathoexcitatory stress responses (Thayer and Sternberg, 2006). Furthermore, our interpretation is consistent with the finding that tonic PNS function is crucial for the dynamical properties of heartbeat interval fluctuations (Stiedl et al., 2009, 2010), as determined by nonlinear analysis.

Considering the very short half-life of metyrapone (approx. 2 h), the profound effects of metyrapone challenge on ANS activity 8–9 h after administration clearly suggest indirect effects of HPA axis stimulation. Given the association of higher cortisol levels with enhanced serotonin reuptake (i.e. lower levels of brain serotonin) (Tafet et al., 2001a, b), as well as the association of lower levels of brain serotonin with reduced HRV (Booij et al., 2006), higher cortisol levels (i.e. HPA axis stimulation) may act indirectly via altered serotonergic signalling to influence the functional connectivity between PFC and the amygdala (Pezawas et al., 2005). Thereby, autonomic and particularly vagal activity (Jordan, 2005; Thayer et al., 2006) is altered via 5-$HT_{1A}$ receptor involvement (Youn et al., 2013). This is also supported by two prior studies of our group, reporting significant effects of both the serotonin transporter-linked polymorphic region (5-HTTLPR) genotype (Agorastos et al., 2014) and long-term treatment with the selective serotonin reuptake inhibitor (SSRI) escitalopram (Agorastos et al., 2015) on stress-related autonomic reactivity, as measured by HRV measures.

It is very important to note that this study is measuring differences at specific time points of the day after nocturnal HPA axis stimulation (i.e. single-time point measurements), which can only reflect indirect effects of the experimental treatment conditions 9 h later. The complex pharmacokinetics of DEX and MET and their yet not fully understood interrelated pharmacodynamics, in addition to the nocturnal circadian phase with evolving different sleep stages and huge changes of HPA axis activity (e.g., nocturnal cortisol rise) and circadian gene expression, represent vital limitations. Our results, thus, represent only a small piece of evidence in the highly complex HPA/ANS interplay, which remains to be further investigated. Future studies could also shed more light to the large number of pathways possibly responsible for the neglectable HRV differences between baseline and post-DEX conditions reported here, in contrast to our initial hypothesis.

Finally, some additional limitations of our study merit discussion. Because of rigid exclusion criteria, the combined endocrine challenge and its time intensive nature, our study investigated only a relatively small number of healthy volunteers. Our findings should, thus, be replicated in larger study populations. On the other hand, all subjects were extremely carefully selected to minimize the probability of medical (e.g., medication use, depression) and behavioural (e.g., substance, tobacco and alcohol use) confounders. It is particularly important to note that across all parameters investigated, no subject had a cardiovascular history and deviating laboratory or physical tests. We particularly accounted for several laboratory markers (e.g., fasting glucose, hemoglobin A1c levels, cholesterol/lipoproteins, pro-inflammatory cytokines, acute-phase proteins) and certain lifestyle habits (e.g., drug, alcohol or tobacco intake) that have been shown to be associated with ANS dysregulation altering cardiovascular measures including HRV (Dinas et al., 2013; Thayer and Sternberg, 2006). With respect to our baseline HRV values, mean RR (NN) and RMSSD were found in the physiological range for healthy humans (Nunan et al., 2010) and only the SDNN values in our study (93–98 ms) slightly exceed the upper range of 93 ms listed by Nunan et al. (2010). This is probably attributed to the supine position of our study volunteers during the ECG recordings, as supine positioning is known to increase HRV when compared to other postures such as sitting or standing (Young and Leicht, 2011). However, as disturbed sleep is also associated with autonomic alterations (Nielsen et al., 2010), it is important to note that we did not objectively measure sleep quality in our initial assessments and can herewith not exclude sleep quality-related bias. In addition, although having excluded actual menstruation, we have not controlled for the specific menstrual phase of women participants. Secondary analyses investigating the impact of peri- and postmenopausal status (2 and 4 women, respectively) on our results, did not alter our findings. Respiration should be added as additional measure in future studies since particularly deep breathing profoundly increases HRV, which is referred to as respiratory sinus arrhythmia. Finally, overall the dynamic ANS and endocrine changes along the time scale from pharmacological intervention to the measurement interval (her after 9 h) need to be checked in future studies to develop a mechanistic understanding of the reported long-term effects.

## 5. Conclusions

Variation in the activity of the stress-responsive ANS and HPA axis is particularly important for adaptive stress responses and may thus give rise to individual differences in resilience as ability to cope with stressful events. Studies about effects of HPA axis activity on baseline autonomic activity have not been reported so far in healthy subjects. By assessing autonomic responses through linear and nonlinear analyses of HRV to endocrine HPA axis challenges, we provide first data that metyrapone treatment is associated with reduced vagal tone, potentially via elevated long-lasting CRH action, while HPA axis suppression has no distinct effect on autonomic activity. Our study supports a vital role of the PNS in the interplay between ANS and HPA axis, as well as in the modulation of stress-related cardiovascular responsiveness. PNS activity may, thus, particularly affect susceptibility to stress-related disorders and represent an important pathway towards the higher cardiac mortality seen in these disorders. In addition, our results underline the utility of HRV as potential biomarker for stress system sensitivity and vulnerability to stress-related disorders. Future studies are needed to replicate this finding and to further explore the functional contribution of PNS on stress responsiveness in order to mechanistically understand its role over SNS in the pathophysiology of stress-related disorders.

## Contributors

AA, CD and KW designed the study and wrote the protocol. AH, CM and AS collected the data. AH managed recruitment and screening of volunteers, as well as all literature searches. AA, AH, OS and TH had access to the raw data, performed all data processing and analyses. AA performed all statistical analyses and interpretation. AA and AH wrote the first draft of the paper. KW, CD, KRS and OS revised the draft for important intellectual content. All authors have contributed to, read and approved the final version of the manuscript.

## Conflict of interest

All authors report no biomedical financial interests or potential conflicts of interest and none of the other authors received funding for this article.

## Role of funding source

None.

## Acknowledgement

This manuscript is part of the doctoral thesis of AH.

## References

Adlan, A.M., Veldhuijzen van Zanten, J., Lip, G.Y.H., Paton, J.F.R., Kitas, G.D., Fisher, J.P., 2018. Acute hydrocortisone administration reduces cardiovagal baroreflex sensitivity and heart rate variability in young men. J. Physiol. (Paris) 20, 4847–4861.

Agorastos, A., Boel, J.A., Heppner, P.S., Hager, T., Moeller-Bertram, T., Haji, U., Motazedi, A., Yanagi, M.A., Baker, D.G., Stiedl, O., 2013. Diminished vagal activity and blunted diurnal variation of heart rate dynamics in posttraumatic stress disorder. Stress 16, 300–310.

Agorastos, A., Kellner, M., Stiedl, O., Muhtz, C., Becktepe, J.S., Wiedemann, K., Demiralay, C., 2014. The 5-HTTLPR genotype modulates heart rate variability and its adjustment by pharmacological panic challenge in healthy men. J. Psychiatr. Res. 50, 51–58.

Agorastos, A., Kellner, M., Stiedl, O., Muhtz, C., Wiedemann, K., Demiralay, C., 2015. Blunted autonomic reactivity to pharmacological panic challenge under long-term escitalopram treatment in healthy men. Int. J. Neuropsychopharmacol. 18.

Agorastos, A., Demiralay, C., Stiedl, O., Muhtz, C., Wiedemann, K., Kellner, M., 2016. Metabotropic glutamate2/3 receptor agonism facilitates autonomic recovery after pharmacological panic challenge in healthy humans. Int. Clin. Psychopharmacol. 31, 176–178.

Akselrod, S., Gordon, D., Ubel, F.A., Shannon, D.C., Berger, A.C., Cohen, R.J., 1981. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 213, 220–222.

Ali, N., Nitschke, J.P., Cooperman, C., Pruessner, J.C., 2017. Suppressing the endocrine and autonomic stress systems does not impact the emotional stress experience after psychosocial stress. Psychoneuroendocrinology 78, 125–130.

Arlt, J., Jahn, H., Kellner, M., Strohle, A., Yassouridis, A., Wiedemann, K., 2003. Modulation of sympathetic activity by corticotropin-releasing hormone and atrial natriuretic peptide. Neuropeptides 37, 362–368.

Aubert, A.E., Vandeput, S., Beckers, F., Liu, J., Verheyden, B., Van Huffel, S., 2009. Complexity of cardiovascular regulation in small animals. Philos. Trans. A Math. Phys. Eng. Sci. 367, 1239–1250.

Avgerinos, P.C., Nieman, L.K., Oldfield, E.H., Cutler Jr., G.B., 1996. A comparison of the overnight and the standard metyrapone test for the differential diagnosis of adrenocorticotrophin-dependent Cushing's syndrome. Clin. Endocrinol. (Oxf.) 45, 483–491.

Baumert, J.H., Frey, A.W., Adt, M., 1995. [Analysis of heart rate variability. Background, method, and possible use in anesthesia]. Anaesthesist 44, 677–686.

Baumert, M., Baier, V., Haueisen, J., Wessel, N., Meyerfeldt, U., Schirdewan, A., Voss, A., 2004. Forecasting of life threatening arrhythmias using the compression entropy of heart rate. Methods Inf. Med. 43, 202–206.

Benarroch, E.E., 1993. The central autonomic network: functional organization, dysfunction, and perspective. Mayo Clin. Proc. 68, 988–1001.

Benjamini, Y., Hochberg, J., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Sci. B 57, 289–300.

Booij, L., Swenne, C.A., Brosschot, J.F., Haffmans, P.M., Thayer, J.F., Van der Does, A.J., 2006. Tryptophan depletion affects heart rate variability and impulsivity in remitted depressed patients with a history of suicidal ideation. Biol. Psychiatry 60, 507–514.

Camm, A.J., Malik, M., Bigger, J.T., Breithardt, G., Cerutti, S., Cohen, R.J., Coumel, P., Fallen, E.L., Kennedy, H.L., Kleiger, R.E., Lombardi, F., Malliani, A., Moss, A.J., Rottman, J.N., Schmidt, G., Schwartz, P.J., Singer, D., 1996. Heart rate variability - standards of measurement, physiological interpretation, and clinical use. Circulation 93, 1043–1065.

Casement, M.D., Goldstein, T.R., Gratzmiller, S.M., Franzen, P.L., 2018. Social stress response in adolescents with bipolar disorder. Psychoneuroendocrinology 91, 159–168.

Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5, 374–381.

Cole, M.A., Kim, P.J., Kalman, B.A., Spencer, R.L., 2000. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 25, 151–167.

Davis, A.M., Natelson, B.H., 1993. Brain-heart interactions. The neurocardiology of arrhythmia and sudden cardiac death. Texas Heart Inst. J. 158–169.

de Kloet, E.R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475.

Dinas, P.C., Koutedakis, Y., Flouris, A.D., 2013. Effects of active and passive tobacco cigarette smoking on heart rate variability. Int. J. Cardiol. 163, 109–115.

Elenkov, I.J., Chrousos, G.P., 2006. Stress system–organization, physiology and immunoregulation. Neuroimmunomodulation 13, 257–267.

Falconer, E., Bryant, R., Felmingham, K.L., Kemp, A.H., Gordon, E., Peduto, A., Olivieri, G., Williams, L.M., 2008. The neural networks of inhibitory control in posttraumatic stress disorder. J. Psychiatry Neurosci. 33, 413–422.

Fiad, T.M., Kirby, J.M., Cunningham, S.K., McKenna, T.J., 1994. The overnight single-dose metyrapone test is a simple and reliable index of the hypothalamic-pituitary-adrenal axis. Clin. Endocrinol. (Oxf.) 40, 603–609.

Goldstein, D.S., 2012. Neurocardiology: therapeutic implications for cardiovascular disease. Cardiovasc. Ther. 30, e89–106.

Gwirtsman, H., Gerner, R.H., Sternbach, H., 1982. The overnight dexamethasone suppression test: clinical and theoretical review. J. Clin. Psychiatry 43, 321–327.

Herman, J.P., 2017. Regulation of hypothalamo-pituitary-adrenocortical responses to stressors by the nucleus of the solitary tract/dorsal vagal complex. Cell. Mol. Neurobiol.

Ikegami, T., Suzuki, K., 2008. From a homeostatic to a homeodynamic self. Biosystems 91, 388–400.

Jarrin, D.C., McGrath, J.J., Giovanniello, S., Poirier, P., Lambert, M., 2012. Measurement fidelity of heart rate variability signal processing: the devil is in the details. Int. J. Psychophysiol. 86, 88–97.

Jordan, D., 2005. Vagal control of the heart: central serotonergic (5-HT) mechanisms. Exp. Physiol. 90, 175–181.

Jose, A.D., Collison, D., 1970. The normal range and determinants of the intrinsic heart rate in man. Cardiovasc. Res. 4, 160–167.

Kiem, S.A., Andrade, K.C., Spoormaker, V.I., Holsboer, F., Czisch, M., Samann, P.G., 2013. Resting state functional MRI connectivity predicts hypothalamus-pituitary-axis status in healthy males. Psychoneuroendocrinology 38, 1338–1348.

Kleiger, R.E., Stein, P.K., Bosner, M.S., Rottman, J.N., 1992. Time domain measurements of heart rate variability. Cardiol. Clin. 10, 487–498.

Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flugge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wohr, M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35, 1291–1301.

Laborde, S., Mosley, E., Thayer, J.F., 2017. Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. Front. Psychol. 8, 213.

Licht, C.M., Vreeburg, S.A., van Reedt Dortland, A.K., Giltay, E.J., Hoogendijk, W.J., DeRijk, R.H., Vogelzangs, N., Zitman, F.G., de Geus, E.J., Penninx, B.W., 2010. Increased sympathetic and decreased parasympathetic activity rather than changes in hypothalamic-pituitary-adrenal axis activity is associated with metabolic abnormalities. J. Clin. Endocrinol. Metab. 95, 2458–2466.

Lingjaerde, O., Ahlfors, U.G., Bech, P., Dencker, S.J., Elgen, K., 1987. The UKU side effect

rating scale. A new comprehensive rating scale for psychotropic drugs and a cross-sectional study of side effects in neuroleptic-treated patients. Acta Psychiatr. Scand. Suppl. 334, 1–100.
Loewy, A.D., Spyer, K.M., 1990. Central Regulation of Autonomic Functions. Oxford University Press, Oxford.
Lombardi, F., Stein, P.K., 2011. Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function. Front. Physiol. 2, 95.
Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445.
McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179.
Meyer, M., 2002. Fractal scaling of heart rate dynamics in health and disease. In: Losa, G.A., Merlini, D., Nonnenmacher, T.F., Weibel, E.R. (Eds.), Fractals in Biology and Medicine. Birkhäuser, Basel, pp. 181–193.
Meyer, M., Stiedl, O., 2003. Self-affine fractal variability of human heartbeat interval dynamics in health and disease. Eur. J. Appl. Physiol. 90, 305–316.
Meyer, M., Stiedl, O., 2006. Fractal rigidity by enhanced sympatho-vagal antagonism in heartbeat interval dynamics elicited by central application of corticotropin-releasing factor in mice. J. Math. Biol. 52, 830–874.
Nielsen, T., Paquette, T., Solomonova, E., Lara-Carrasco, J., Colombo, R., Lanfranchi, P., 2010. Changes in cardiac variability after REM sleep deprivation in recurrent nightmares. Sleep 33, 113–122.
Nijsen, M.J., Croiset, G., Diamant, M., Stam, R., Kamphuis, P.J., Bruijnzeel, A., de Wied, D., Wiegant, V.M., 2000a. Endogenous corticotropin-releasing hormone inhibits conditioned-fear-induced vagal activation in the rat. Eur. J. Pharmacol. 389, 89–98.
Nijsen, M.J., Croiset, G., Stam, R., Bruijnzeel, A., Diamant, M., de Wied, D., Wiegant, V.M., 2000b. The role of the CRH type 1 receptor in autonomic responses to corticotropin- releasing hormone in the rat. Neuropsychopharmacology 22, 388–399.
Nijsen, M.J., Croiset, G., Diamant, M., De Wied, D., Wiegant, V.M., 2001. CRH signalling in the bed nucleus of the stria terminalis is involved in stress-induced cardiac vagal activation in conscious rats. Neuropsychopharmacology 24, 1–10.
Nonell, A., Bodenseh, S., Lederbogen, F., Kopf, D., Hamann, B., Gilles, M., Deuschle, M., 2005. Chronic but not acute hydrocortisone treatment shifts the response to an orthostatic challenge towards parasympathetic activity. Neuroendocrinology 81, 63–68.
Nunan, D., Sandercock, G.R., Brodie, D.A., 2010. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. Pacing Clin. Electrophysiol. 33, 1407–1417.
Oliveira, L.A., Almeida, J., Benini, R., Crestani, C.C., 2015. CRF1 and CRF2 receptors in the bed nucleus of the stria terminalis modulate the cardiovascular responses to acute restraint stress in rats. Pharmacol. Res. 95-96, 53–62.
Peng, C.K., Havlin, S., Stanley, H.E., Goldberger, A.L., 1995. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos 5, 82–87.
Pervanidou, P., 2008. Biology of post-traumatic stress disorder in childhood and adolescence. J. Neuroendocrinol. 20, 632–638.
Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nat. Neurosci. 8, 828–834.
Pulopulos, M.M., Vanderhasselt, M.A., De Raedt, R., 2018. Association between changes in heart rate variability during the anticipation of a stressful situation and the stress-induced cortisol response. Psychoneuroendocrinology 94, 63–71.
Quaedflieg, C.W., van de Ven, V., Meyer, T., Siep, N., Merckelbach, H., Smeets, T., 2015. Temporal dynamics of stress-induced alternations of intrinsic amygdala connectivity and neuroendocrine levels. PLoS One 10, e0124141.
Raison, C.L., Miller, A.H., 2003. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am. J. Psychiatry 160, 1554–1565.
Reyes del Paso, G.A., Langewitz, W., Mulder, L.J., van Roon, A., Duschek, S., 2013. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. Psychophysiology 50, 477–487.
Sakaki, M., Yoo, H.J., Nga, L., Lee, T.H., Thayer, J.F., Mather, M., 2016. Heart rate variability is associated with amygdala functional connectivity with MPFC across younger and older adults. Neuroimage 139, 44–52.
Saper, C.B., 2004. Central autonomic system. In: Paxinos, G. (Ed.), The Rat Nervous System, 3rd ed. Elsevier, Amsterdam, pp. 761–796.
Schmidt, R.F., Thews, G., Lang, F., 2000. Physiologie Des Menschen. Springer, Berlin.
Smeets, T., 2010. Autonomic and hypothalamic-pituitary-adrenal stress resilience: impact of cardiac vagal tone. Biol. Psychol. 84, 290–295.
Stiedl, O., Meyer, M., Jahn, O., Ogren, S.O., Spiess, J., 2005. Corticotropin-releasing factor receptor 1 and central heart rate regulation in mice during expression of conditioned fear. J. Pharmacol. Exp. Ther. 312, 905–916.
Stiedl, O., Jansen, R.F., Pieneman, A.W., Ogren, S.O., Meyer, M., 2009. Assessing aversive emotional states through the heart in mice: implications for cardiovascular dysregulation in affective disorders. Neurosci. Biobehav. Rev. 33, 181–190.
Stiedl, O., Youn, J., Jansen, R.F., 2010. Cardiovascular conditioning: neural substrates. In: Koob, G.F., LeMoal, M., Thompson, R.F. (Eds.), Encyclopedia of Behavioral Neuroscience. Elsevier, Amsterdam, pp. 226–235.
Stratakis, C.A., Chrousos, G.P., 1995. Neuroendocrinology and pathophysiology of the stress system. Ann. N. Y. Acad. Sci. 771, 1–18.
Tafet, G.E., Idoyaga-Vargas, V.P., Abulafia, D.P., Calandria, J.M., Roffman, S.S., Chiovetta, A., Shinitzky, M., 2001a. Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. Cogn. Affect. Behav. Neurosci. 1, 388–393.
Tafet, G.E., Toister-Achituv, M., Shinitzky, M., 2001b. Enhancement of serotonin uptake by cortisol: a possible link between stress and depression. Cogn. Affect. Behav. Neurosci. 1, 96–104.
Thayer, J.F., 2006. On the importance of inhibition: central and peripheral manifestations of nonlinear inhibitory processes in neural systems. Dose. 4, 2–21.
Thayer, J.F., Lane, R.D., 2000. A model of neurovisceral integration in emotion regulation and dysregulation. J. Affect. Disord. 61, 201–216.
Thayer, J.F., Lane, R.D., 2009. Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. Neurosci. Biobehav. Rev. 33, 81–88.
Thayer, J.F., Sternberg, E., 2006. Beyond heart rate variability: vagal regulation of allostatic systems. Ann. N. Y. Acad. Sci. 1088, 361–372.
Thayer, J.F., Hall, M., Sollers 3rd., J.J., Fischer, J.E., 2006. Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: evidence for impaired inhibitory control of the HPA axis in heavy drinkers. Int. J. Psychophysiol. 59, 244–250.
Thayer, J.F., Ahs, F., Fredrikson, M., Sollers 3rd., J.J., Wager, T.D., 2012. A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. Neurosci. Biobehav. Rev. 36, 747–756.
Tovote, P., Meyer, M., Ronnenberg, A., Ogren, S.O., Spiess, J., Stiedl, O., 2005. Heart rate dynamics and behavioral responses during acute emotional challenge in corticotropin-releasing factor receptor 1-deficient and corticotropin-releasing factor-overexpressing mice. Neuroscience 134, 1113–1122.
Tran, L., Schulkin, J., Greenwood-Van Meerveld, B., 2014. Importance of CRF receptor-mediated mechanisms of the bed nucleus of the stria terminalis in the processing of anxiety and pain. Neuropsychopharmacology 39, 2633–2645.
Tsigos, C., Chrousos, G.P., 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J. Psychosom. Res. 53, 865–871.
Ulrich-Lai, Y.M., Herman, J.P., 2009. Neural regulation of endocrine and autonomic stress responses. Nat. Rev. Neurosci. 10, 397–409.
Veer, I.M., Oei, N.Y., Spinhoven, P., van Buchem, M.A., Elzinga, B.M., Rombouts, S.A., 2012. Endogenous cortisol is associated with functional connectivity between the amygdala and medial prefrontal cortex. Psychoneuroendocrinology 37, 1039–1047.
Verhoeven, K.J.F., Simonsen, K.L., McIntyre, L.M., 2005. Implementing false discovery rate control: increasing your power. Oikos 108, 643–647.
Weber, C.S., Thayer, J.F., Rudat, M., Wirtz, P.H., Zimmermann-Viehoff, F., Thomas, A., Perschel, F.H., Arck, P.C., Deter, H.C., 2010. Low vagal tone is associated with impaired post stress recovery of cardiovascular, endocrine, and immune markers. Eur. J. Appl. Physiol. 109, 201–211.
Wiedemann, K., Jahn, H., Yassouridis, A., Kellner, M., 2001. Anxiolyticlike effects of atrial natriuretic peptide on cholecystokinin tetrapeptide-induced panic attacks: preliminary findings. Arch. Gen. Psychiatry 58, 371–377.
Wood, S.K., Woods, J.H., 2007. Corticotropin-releasing factor receptor-1: a therapeutic target for cardiac autonomic disturbances. Expert Opin. Ther. Targets 11, 1401–1413.
Yamamoto, Y., Nakamura, Y., Sato, H., Yamamoto, M., Kato, K., Hughson, R.L., 1995. On the fractal nature of heart rate variability in humans: effects of vagal blockade. Am. J. Physiol. 269, R830–837.
Youn, J., Hager, T., Misane, I., Pieneman, A.W., Jansen, R.F., Ogren, S.O., Meyer, M., Stiedl, O., 2013. Central 5-HT receptor-mediated modulation of heart rate dynamics and its adjustment by conditioned and unconditioned fear in mice. Br. J. Pharmacol.
Young, F.L., Leicht, A.S., 2011. Short-term stability of resting heart rate variability: influence of position and gender. Appl. Physiol. Nutr. Metab. 36, 210–218.