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RESEARCH ARTICLE

Effect of chronic stress on cardiovascular and ventilatory responses activated by both chemoreflex and baroreflex in rats

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ABSTRACT

Chronic stress results in physiological and somatic changes. It has been recognized as a risk factor for several types of cardiovascular dysfunction and changes in autonomic mechanisms, such as baroreflex and chemoreflex activity. However, the effects of different types of chronic stress on these mechanisms are still poorly understood. Therefore, in the present study, we investigated, in adult male rats, the effect of repeated restraint stress (RRS) or chronic variable stress (CVS) on baroreflex, chemoreflex and heart rate variability in a protocol of 14 days of stress sessions. Exposure to RRS and CVS indicated no changes in the basal level of either arterial pressure or heart rate. However, RRS and CVS were able to attenuate sympathovagal modulation and spontaneous baroreflex gain. Additionally, only RRS was able to increase the power of the low-frequency band of the systolic blood pressure spectrum, as well as the slope of linear regression of baroreflex bradycardic and tachycardic responses induced by vasoactive compounds. Additionally, our study is one of the first to show that exposure to RRS and CVS decreases the magnitude of the pressor response and potentiates respiratory responses to chemoreflex activation, which can trigger cardiovascular and respiratory pathologies. Furthermore, the basal respiratory parameters, such as minute ventilation and tidal volume, were significantly decreased by both protocols of chronic stress. However, only CVS increased the basal respiratory frequency. In this way, the findings of the present study demonstrate the impact of chronic stress in terms of not only depressive-like behavior but also alterations of the autonomic baroreflex responses and cardiocirculatory variability (systolic blood pressure and pulse interval).Our results provide evidence that chronic stress promotes autonomic dysregulation, and impairment of baroreflex, chemoreflex and heart rate variability.

KEY WORDS: Chronic stress, Baroreflex, Chemoreflex, Heart rate variability, Autonomic dysregulation, Variable chronic stress, Repeated chronic stress

INTRODUCTION

Living organisms are constantly subjected to a wide range of stressful stimuli that affect many physiological processes (Marin et al., 2007). Stress can affect the function of the immune system by modulating processes in the central nervous system Nakata et al. (1993); moreover, stress can alter and activate endocrine processes associated with the hypothalamus, pituitary and adrenal, and adrenergic system (Lupien and McEwen, 1997; Yaribeygi et al., 2017). In addition, changes in cardiovascular variables, such as blood pressure (BP) and heart rate (f_H), also occur. Because of this, psychological stress has been recognized as a risk factor for several cardiovascular dysfunctions (Inoue, 2014). Although BP and f_H changes are common after exposure to stress, they can vary according to different stressors (Crestani et al., 2010; Crestani, 2016).

Studies of the physiological effects of stress have commonly used different psychological stress protocols, involving daily exposure to the same type of stressor (homotypic) or different aversive stimuli (heterotypic) (Magariños and McEwen, 1995; Crestani, 2016; Duarte et al., 2015a; Vieira et al., 2018). In repeated restraint stress (RRS) models, animals are subjected to the homotypic stressor, while in chronic variable stress (CVS) models, animals are subjected to heterotypic stressors to investigate physiological, behavioral and somatic responses to stress (Ulrich-Lai and Herman, 2009; Grippo et al., 2002; Magariños and McEwen, 1995; Willner, 1997). Additionally, some studies have shown that RRS and CVS in rats may have a different impact on their behavior, body weight and plasma corticosterone secretion (Marin et al., 2007; Ortiz et al., 1996; Martí et al., 1994; Crestani, 2016).

Psychological stress can affect the body through two main pathways: the hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (Kim et al., 2018; Marques et al., 2010). It is known that one of the main endocrine responses to stress is activation of the HPA axis, which results in a rapid increase in circulating glucocorticoids (Aguilera, 1998; Levine, 2005). Both physiological and psychological stress activate the HPA axis, resulting in the release of corticotrophin releasing hormone by the hypothalamus in the hypothalamic–pituitary circulation, thereby stimulating the release of adrenocorticotropic hormone (ACTH) from the pituitary into the general circulation. This in turn stimulates systemic release of glucocorticoids by the cortex of the adrenal gland (Myers et al., 2014; Kolber et al., 2008). In the same way, the autonomic nervous system quickly promotes physiological changes through the sympathetic nervous system and parasympathetic nervous system, via a decrease of the parasympathetic response and activation of the sympathetic response (Porges, 1995).These changes are followed by several alterations in the central nervous system, including the release of noradrenaline from the locus coeruleus (Curtis et al., 1997).

Chronic stress protocols are capable of modifying HPA axis activity, besides stimulating separate regions of the nervous system to elicit appropriate responses (Scharf and Schmidt, 2012). A study performed by Cruz et al. (2012) showed that animals subjected to RRS present a habituation in the release of corticosterone in response to a new acute exposure to restraint stress, whereas animals

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List of abbreviations

ACTH	adrenocorticotropic hormone
BP	blood pressure
BP_{50}	median blood pressure
COPD	chronic obstructive pulmonary disease
CVS	chronic variable stress
f_H	heart rate
f_R	respiratory frequency
HF	high frequency
HPA	hypothalamic–pituitary–adrenal
HRV	heart rate variability
LF	low frequency
MAP	mean arterial pressure
PI	pulse interval
RRS	repeated restraint stress
SAP	systolic arterial pressure
V_E	minute ventilation
V_T	tidal volume

exposed to CVS presented a sustained elevation in corticosterone levels (Magariños and McEwen, 1995; Gadek-Michalska and Bugajski, 2003). However, chronic activation of the HPA axis is capable of promoting physiological changes, resulting in hypertension, immunosuppression and behavioral changes (McEwen, 2000; McEwen et al., 2012). In addition, cardiovascular alterations caused by chronic stress are followed by modifications in baroreflex activity (Duarte et al., 2015a), a neuronal mechanism that is responsible for maintaining the BP at homeostatic levels by influencing variables such as f_H. Studies have demonstrated that adult rodents exposed to CVS had impairment in baroreflex activity (Grippo et al., 2008). Nevertheless, modifications in baroreflex activity and autonomic imbalance have also been reported in adult rats exposed to RRS (Haibara et al., 1999). According to Engi et al. (2012), impairment of baroreflex activity may contribute to the pathogenesis of various cardiovascular diseases. In this context, efficient reversal of stress-induced autonomic changes may be important to reduce the chance of long-term cardiovascular pathologies (Crestani et al., 2010).

In addition to the baroreflex, the chemoreflex is a relevant neural mechanism responsible for evoking respiratory and cardiovascular responses as a result of specific hypoxic or hypercapnia conditions (Fitzgerald, 2000). A widely used animal model of acute and transient cytotoxic hypoxia is the systemic infusion of low doses of potassium cyanide (KCN) (Haibara et al., 1995; Olivan et al., 2001; Kuntze et al., 2016; Granjeiro et al., 2011). The physiological responses characteristic of activation of the chemoreflex, using such a model, is tachypnea accompanied by a BP rise and bradycardia (Franchini and Krieger, 1993; Haibara et al., 1999; Fernandes et al., 2005; Kuntze et al., 2016), followed by behavioral responses characterized by arousal and increased exploration of the environment (Franchini and Krieger, 1993).

Some studies have shown that stress interferes with respiratory activity and that early stress exposure alters the developmental trajectory of the respiratory control system (Bavis and Mitchell, 2008; Cayetanot et al., 2009). According to Genest et al. (2004), maternal separation in early life induces ventilatory alterations in rats, such as altered minute ventilation (V_E) and increased BP. Studies have also reported that animals undergoing neonatal stress have similar respiratory and cardiovascular changes to those in patients who have obstructive sleep syndrome, and become more sensitive to chemoreflex activation (Genest et al., 2004; Gulemetova et al., 2013). Most of the studies cited above demonstrate the effect of stress early in life or in adolescent animals. However, there are no data in the literature demonstrating the effect of the different types of stress in the autonomous baroreflex and chemoreflex mechanisms of adult animals.

According to the evidence of cardiovascular and autonomic changes induced by chronic stressors, the hypothesis of the present study was that different types of chronic stress (RRS and CVS) in adult animals alter the cardiovascular and ventilatory responses controlled by the autonomic system such as the baroreflex and chemoreflex, as well as interfering with heart rate variability. Based on this, our purpose was to investigate the cardiovascular and respiratory consequences of exposure to RRS and CVS in adult animals.

MATERIALS AND METHODS

Animals

A total of 51 adult male Wistar rats (aged 60 days) were used in the present study. All animals were kept in the Animal Care Unit of the School of Medicine of Ribeirão Preto, University of São Paulo Ribeirão Preto (USP). The animals were housed in groups of five per cage in a temperature-controlled room (24±1°C) under a 12 h light/dark cycle (lights on at 06:00 h) and were given water and food *ad libitum*. All experiments were performed in accordance with Ethical Principles for Animal Experimentation followed by the Brazilian Committee for Animal Experimentation (COBEA) and approved by the Committee of Ethics in Animal Research of the School of Medicine of Ribeirão Preto, University of São Paulo (number 175/2014).

Chronic stress regimens

A schematic representation of the complete protocol is presented in Fig. 1. The chronic stress regimens were based on protocols previously used and modified accordingly (Marin et al., 2007; Cruz et al., 2012; Duarte et al., 2015b). Two animal models of stress were chosen. RRS was used as a homotypic stressor, whereas CVS was used as a heterotypic stressor. A total of 31 animals were submitted to stress sessions. Animals of the RRS group were restrained in plastic cylindrical tubes, 17 cm long and 7.5 cm high, for 1 h daily starting at 10:00 h for 14 consecutive days. Animals of the CVS group were exposed to different stressors on variable schedules for 14 consecutive days (Table 1). The protocols were initiated 7 days apart from each other, so that the experiments performed with the respective groups also complied with this interval. Animals of the control group were left undisturbed, except for cleaning of the cages. All tests described below, except for the behavioral tests, were carried out on the same animals.

Experimental protocols

Adult animals were randomly divided into three groups: (1) control, (2) RRS and (3) CVS; the mass of the animals was measured every

Fig. 1. Schematic representation of chronic variable stress (CVS) and repeated restraint stress (RRS) protocols. All animals of CVS and RRS groups were subjected to daily sessions of stress from postnatal day (PND) 60 to 74. After the last session of stress, animals underwent surgical preparation and cardiovascular recording was performed 24 h later.

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Table 1. Chronic variable stress (CVS) protocol

Day	Stress type and schedule
1	10:00 h restraint stress, 60 min; 19:00 h, humid sawdust, overnight.
2	03:00 h, cold (4°C) isolation, 60 min; 19:00 h, lights on, overnight
3	12:00 h, lights off, 180 min; 15:00 h, swim stress, 4 min
4	07:30 h, humid sawdust, all day; 19:00 h, food/water deprivation, overnight
5	13:00 h, swim stress, 3 min; 19:00 h, isolation housing, overnight
6	14:00 h, cold (4°C) isolation, 15 min; 15:00 h, lights off, 120 min
7	19:00 h, humid sawdust and lights on, overnight
8	19:00 h, isolation and food/water deprivation, overnight
9	16:00 h, restraint stress, 60 min; 19:00 h, lights on, overnight
10	09:00 h, swim stress, 4 min; 10:00 h, restraint stress, 60 min
11	03:00 h, cold (4°C) isolation, 60 min; 19:00 h, food/water deprivation, overnight
12	10:00 h restraint stress, 60 min; 19:00 h, humid sawdust, overnight
13	07:30 h, humid sawdust, all day; 19:00 h, food/water deprivation, overnight
14	10:00 h restraint stress, 60 min

day before the stress protocol. Animals of the CVS and RRS groups were subjected to daily sessions of stress for 14 consecutive days. On the 14th day, after the last session of stress, animals in all experimental groups were prepared for surgery; the tests were performed 24 h later. On test days, animals were transferred to the experimental room in their home box and allowed to adapt for 30 min to experimental room conditions before experiments began. In the sequence, a 10 min period of basal cardiovascular activity was recorded for power spectral analysis and spontaneous baroreflex sensitivity. Afterwards, we performed the baroreflex and chemoreflex stimulation following the methods mentioned above (Fig. 1).

Sucrose preference

This test was developed with an independent group of 20 animals. At the end of the chronic stress protocol, ingestion of sucrose (2% solution) was evaluated as a measure of anhedonia (decrease in or absence of the ability to experience pleasure) through a preference test with sucrose. Therefore, the protocol was carried out over 2 days. On each day, the animals were treated individually. On the first day, individual animals were habituated to samples for a period of 5 h. The following day (testing day), the two bottles containing either pure water or sucrose solution were first weighed according to protocol (initial mass) (Willner, 1997) then offered to rats for a period of 10 h; they were then weighed again (final mass). Consumption of both water and sucrose solution was calculated as the difference between initial and final mass. The preference for sucrose was calculated as a percentage by dividing sucrose consumption (in grams) by total consumption (i.e. water consumption+sucrose consumption in grams) and multiplying it by 100.

Surgical procedures

Animals were anesthetized with tribromoethanol (250 mg kg^{-1}, i.p.) and a catheter (a 4 cm segment of PE-10 that was heat-bound to a 13 cm segment of PE-50; Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery for BP recording. A second catheter was implanted into the femoral vein for vasoactive drug infusion. Both catheters were tunneled under the skin and exteriorized on the animal's dorsum. After surgery, the animals were given a polyantibiotic preparation of streptomycin and penicillin (0.3 ml, i.m.; Pentabiotic, 80,000 IU; Fort Dodge, Campinas, SP, Brazil) to prevent infection and also received the non-steroidal anti-inflammatory flunixine meglumine (2.5 mg kg^{-1}, s.c., Banamine; Intervet Schring-Plough, Cruzeiro, SP, Brazil) for post-operative care.

Measurement of cardiovascular parameters

The pulsatile arterial pressure of freely moving animals was recorded using an ML870 preamplifier (LabChart) and an acquisition board (PowerLab, ADInstruments, Bella Vista, NSW, Australia) connected to a computer. Mean arterial pressure (MAP) and heart rate (f_H) values were derived from pulsatile recordings and processed on-line.

Power spectral analysis of systolic arterial pressure and pulse interval

Power spectral analysis of systolic arterial pressure (SAP) and pulse interval (PI) was employed to analyze autonomic activity controlling the blood vessels and heart, respectively. For this purpose, relatively stable 10 min duration segments of the pulsatile arterial pressure signals were used in order to extract PI and SAP. Using fast Fourier transform spectral analysis (Software Cardioseries v2.4, https://www.sites.google.com/site/cardioseries/home), the overall variability of these series was calculated in the time and frequency domain. The time domain analysis consisted of calculating the variance in the entire time series. For frequency domain analysis, the PI spectra were integrated into low (LF: 0.20–0.75 Hz) and high (HF: 0.75–3.00 Hz) frequency bands, whereas the SAP spectra were integrated only into the LF band (0.20–0.75 Hz).

Assessment of spontaneous baroreflex sensitivity

The baroreflex sensitivity was calculated in the time domain as follows. In the time series, the SAP and PI were processed by Cardioseries v2.4 software (https://www.sites.google.com/site/cardioseries/home). For this, beat to beat values of SAP and PI were analyzed for identification of sequences in which SAP increases were associated with PI lengthening (up sequence) or SAP decreases were associated with PI shortening (down sequence). Differences were considered only when changes greater than or equal to 1 mmHg (SAP) and 1 ms (PI) were observed. The spontaneous baroreflex sensitivity was determined using the average slope of the linear regression between the SAP and PI values found for each sequence.

Baroreflex assessment

The baroreflex was activated by phenylephrine (α1-adrenoceptor agonist; 50 µg kg^{-1}, 0.34 ml min^{-1}) or sodium nitroprusside (SNP; NO donor; 50 µg kg^{-1}, 0.8 ml min^{-1}) infusion using an infusion pump (KD Scientific, Holliston, MA, USA). The phenylephrine or SNP infusion lasted 30–40 s and caused, respectively, an increase and a decrease in BP (Alves et al., 2009).

Evaluation of baroreflex activity

Baroreflex curves were constructed by matching variation in MAP (ΔMAP) with f_H responses (Δf_H). Paired values for ΔMAP and Δf_H were plotted to create sigmoid curves for each rat, which were used to determine baroreflex activity (Resstel et al., 2004). To analyze bradycardic and tachycardic responses separately, f_H values matching 10, 20, 30 and 40 mmHg of MAP changes were calculated (Alves et al., 2009). Values were plotted to create linear regression curves for each rat, and their slopes were compared to determine changes in baroreflex gain.

Chemoreflex activation

The chemoreflex was activated by systemic bolus injections of 0.1 ml KCN i.v. (40 µg per rat; Merck, Darmstadt, Germany) following procedures described by Franchini and Krieger (1993) and experimental conditions previously validated (Granjeiro et al., 2011).

Assessment of cardiovascular parameters during chemoreflex activation

The magnitude of changes in MAP and f_H in response to chemoreflex activation was quantified at the peak, and the duration of the responses was recorded (Granjeiro et al., 2011; Kuntze et al., 2016).

Assessment of respiratory response

The respiratory frequency (f_R) response induced by chemoreflex activation was evaluated by the whole-body plethysmography method for small animals described by Bartlett and Tenney (1970). Rats were kept inside a 6 l Plexiglas® chamber (with sealed exit ports for catheters) which allowed them to move freely. After the chamber was closed for f_R recording, pressure oscillations originated by breathing movements were detected by a differential pressure transducer (ML141 Spirometer, PowerLab, ADInstruments). f_R was expressed as the number of cycles per minute, which was calculated from the extrapolation of the plestimographic signal, regarding the volume calibration accomplished for each measurement (1 ml injection of air into the chamber). Respiratory cycles were acquired 20 s before and 20 s after chemoreflex activation with KCN (i.v.) and were divided into 2 s intervals by the acquisition software program (PowerLab, ADInstruments) with manual corrections, when necessary. Equations described by Drorbaugh and Fenn (1955) allowed calculation of the tidal volume (V_T), and V_E was obtained as the product of f_R and V_T. The V_T and V_E parameters were only calculated 10 points before chemoreflex activation (where the 10 points correspond to 20 s prior to chemoreflex activation with KCN, divided into 2 s intervals for analysis) to avoid incorrect values given that vigorous behavioral responses are evoked by KCN injection.

Data analysis

All the data presented a normal distribution and homogeneity of variance. Data are expressed as means±s.e.m. Results were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test for identification of differences between the groups from GraphPad Prism software version 7 (San Diego, CA, USA). Comparison of the effect of stress on body mass was realized using two-way ANOVA, with stress (control, RRS and CVS) as independent factors, and time as a repeated measurement. Results of statistical tests with $P<0.05$ were considered significant.

RESULTS

Effects of chronic stress on sucrose preference test

The animals subjected to CVS and RRS had a lower preference for sucrose solution when compared with the control group (control 97.31±0.66%, RRS 81.13±5.84%, CVS 78.39±4.05%; $F_{2,17}=7.07$, $P<0.05$) (Fig. 2A).

Effects of chronic stress on body mass and basal cardiovascular parameters

Body mass

Analysis of body mass revealed that animals on the RRS and CVS protocols presented a reduction in body mass when compared with control groups (control 400±20.76 g, RRS 375±15.02 g, CVS 376.7±14.05 g; $F_{2,28}=9.298$, $P<0.05$). Similarly, there was a main effect of time ($F_{2,56}=267.9$, $P<0.05$), especially from the 8th day of stress (Fig. 2B).

Basal cardiovascular parameters

There was no change in basal levels of either MAP ($F_{2,30}=0.2671$, $P>0.05$) or f_H ($F_{2,28}=0.05448$, $P>0.05$) between the different groups (Fig. 3).

Effects of chronic stress in power spectral analysis of SAP and PI

PI

We found no difference in PI ($F_{2,28}=0.7181$, $P>0.05$) and in total PI variability ($F_{2,28}=0.8925$, $P>0.05$) among the stress and control groups (Fig. 4A,B). However, RRS and CVS were able to attenuate both LF ($F_{2,28}=7.883$, $P<0.05$) and HF band power ($F_{2,28}=7.299$; $P<0.05$) (Fig. 4C,D). In addition, it was verified that chronic stress was able to decrease the power of the LF bands ($F_{2,28}=5.280$, $P<0.05$), increase the power of the HF bands ($F_{2,28}=5.280$, $P<0.05$) (Fig. 4E,F) and decrease the band power ratio when compared with the control group ($F_{2,28}=4.307$, $P<0.05$) (Fig. 4G).

SAP

We observed no difference in SAP ($F_{2,28}=1.267$, $P>0.05$) or in total SAP variability ($F_{2,28}=0.3351$, $P>0.05$) among the groups (Fig. 5A,B). The RRS group showed an increase in LF band power ($F_{2,28}=3.488$, $P<0.05$) when compared with the control group (Fig. 5C).

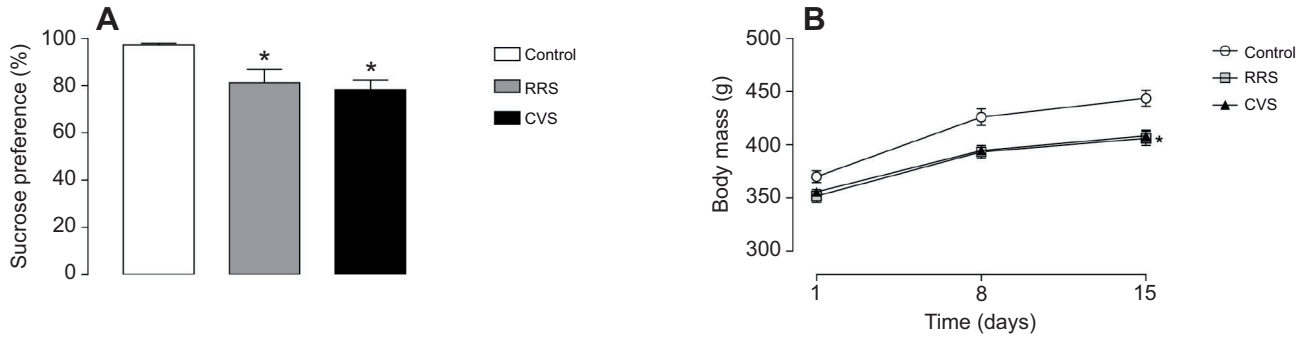

Fig. 2. Sucrose preference test and body mass in control and stressed rats. (A) Consumption of 2% sucrose solution. Means±s.e.m. of control (n=7), RRS (n=6) and CVS (n=7) groups (*$P<0.05$ versus control group, one-way ANOVA followed by Bonferroni's *post hoc* test). (B) Time course of body mass change. Means±s.e.m. of control (n=11), RRS (n=10) and CVS (n=10) groups (*$P<0.05$ versus control group, two-way ANOVA followed by Bonferroni's *post hoc* test).

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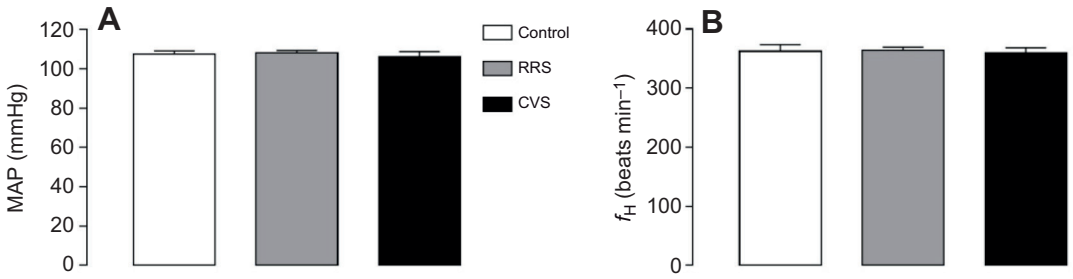

Fig. 3. Basal cardiovascular parameters. (A) Baseline mean arterial pressure (MAP) and (B) baseline heart rate (f_H) in control, RRS and CVS rats. The bars represent means±s.e.m. of the control (n=11), RRS (n=10) and CVS (n=10) groups. *P<0.05 was considered statistically different (one-way ANOVA followed by Bonferroni's *post hoc* test).

Effects of chronic stress in spontaneous baroreflex sensitivity

Baroreflex activity assessed by the sequence analysis technique indicated that both RRS and CVS caused a reduction in total gain ($F_{2,28}$=12.77, P<0.05) (Fig. 6A). In addition, chronic stress also decreased the number of up sequences ($F_{2,28}$=12.74, P<0.05) and the number of down sequences ($F_{2,28}$=6.024, P<0.05) (Fig. 6B,C).

Effects of chronic stress on cardiac baroreflex activity

RRS increased the slope of the linear regression of bradycardic (control −2.07±0.25, RRS −2.99±0.35; $F_{2,25}$=4.090, P<0.05) and tachycardic responses (control −1.59±0.18, RRS=−2.45±0.34; $F_{2,25}$=3.407, P<0.05) (Fig. 7A). However, there was no significant difference between the CVS group and the control group in bradycardic (control −2.07±0.25, CVS −2.04±0.17) and tachycardic responses (control −1.59±0.18, CVS −1.46±0.27). Apart from BP_{50} (median blood pressure, which is the value of MAP when 50% of the f_H is altered), the sigmoid curve parameters (gain, lower and upper f_H plateau and f_H plateau range) were increased in the RRS group (Table 2).

Effects of chronic stress on cardiovascular and ventilatory responses to chemoreflex activation

Cardiovascular responses

The RRS and CVS groups showed a decrease in the magnitude of the pressor response to chemoreflex activation when compared with the control group ($F_{2,28}$=10.32, P<0.05) (Fig. 8A). However, there was no significant difference between groups regarding the bradycardic response ($F_{2,28}$=1.948, P>0.05) (Fig. 8B).

Ventilatory responses

Both chronic stress groups showed a greater average maximum increase of f_R due to activation of the chemoreflex when compared with the control group ($F_{2,28}$=5.317, P<0.05) (Fig. 8C). Regarding the baseline ventilatory parameters, recorded before chemoreflex activation, both the RRS and CVS groups presented attenuation in the V_T ($F_{2,28}$=8.154, P<0.05; Fig. 8D) and V_E ($F_{2,28}$=6.411, P<0.05; Fig. 8E) response when compared with the control group. Additionally, basal f_R was increased only in the CVS group ($F_{2.28}$=4365, P<0.05) (Fig. 8F).

DISCUSSION

The hypothesis of the present study was that different types of chronic stress (RRS and CVS) alter the cardiovascular and ventilatory responses controlled by the autonomic system such as the baroreflex and chemoreflex, as well as interfering in heart rate variability. Our main findings were: (1) RRS but not CVS was able to potentiate tachycardic and bradycardic responses to baroreflex activation induced by vasoactive drugs; (2) chronic stress decreased the variability of PI and spontaneous baroreflex sensitivity and decreased the magnitude of the chemoreflex-induced BP rise; and (3) RRS and CVS decreased the basal respiratory V_T and V_E parameters.

Our results also corroborate with the literature data, which demonstrate that animals subjected to chronic stress protocols (RRS and CVS) present a lower body mass gain over time when compared with control animals that did not receive any type of stress. In addition, both protocols promoted a decrease in the sucrose preference test. The preference for sucrose has been used as a marker of chronic stress exposure in over 50 studies, indicating a depressed-like behavior in animals (Willner, 2005).

In addition to these changes in mass and behavior, several studies have shown that stress can modify the circulatory system activity, depending on the type of stressor involved (Esch et al., 2002; Crestani, 2016; Duarte et al., 2015b), as stress exposure increases MAP and f_H (Grippo et al., 2008; Tavares and Correa, 2006; Irvine et al., 1997; Carrive, 2006; Barr; et al., 2004; Duarte et al., 2015b). Some studies compared the impact on arterial pressure in RRS versus CVS and observed a small pressor effect after 10 days. However, our findings demonstrate that chronic stress was not able to change the baseline parameters of MAP and f_H, which complies with previous observations (Grippo et al., 2006; Grippo et al., 2003). According to Crestani (2016), cardiovascular dysfunction induced by chronic stressors may be influenced by age; thus, differences in rat strains and age could explain these discrepancies.

In addition, cardiovascular alterations caused by chronic stress may reflect baroreflex impairment (Duarte et al., 2015a). Changes in the baroreflex were reported after exposure of different chronic stressors (Almeida et al., 2015; Cruz et al., 2012; Duarte et al., 2015a; Crestani, 2016). Based on this, our results demonstrated that RRS but not CVS was able to increase the magnitude of both tachycardic and bradycardic components of the cardiac baroreflex. Therefore, there was a greater variation of the f_H in response BP variation induced by RRS. However, the effect of chronic stress on the baroreflex seems to be dependent on the duration and type of stress. Differently from our results, in another study, a CVS protocol for 14 days modified baroreflex activity, but this protocol was performed in adolescent animals (Almeida et al., 2015). Other studies have shown that the 4 week CVS protocol did not modify baroreflex activity in adult animals (Grippo et al., 2008; Simas et al., 2018), corroborating our findings. Few studies to date have investigated the influence of predictability by directly comparing cardiovascular and autonomic changes induced by predictable versus unpredictable stress protocols (Almeida et al.,

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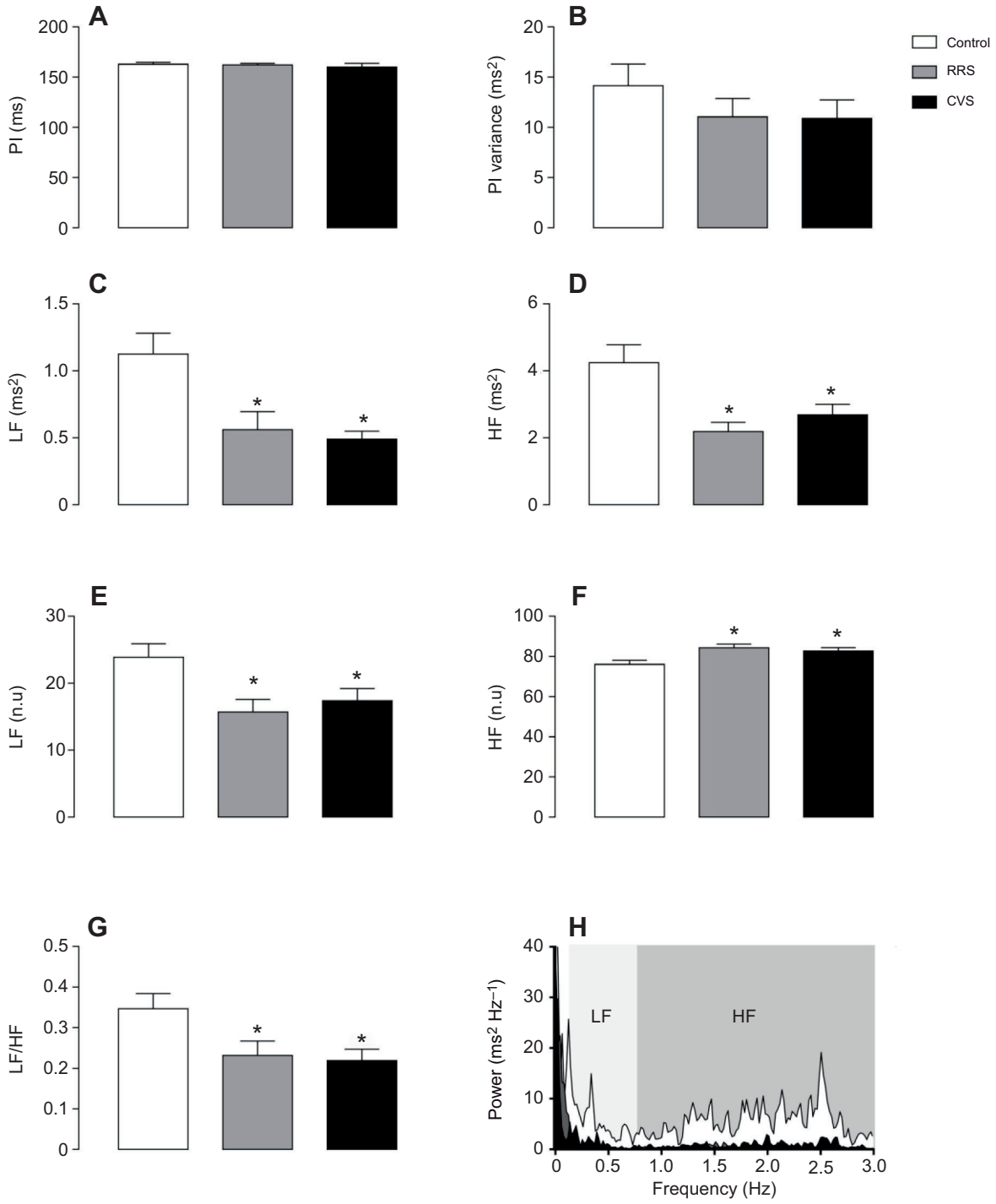

Fig. 4. Effects of chronic stress on heart rate variability (HRV). (A) Pulse interval (PI). (B) Total PI variability. (C,D) Low frequency (LF; C) and high frequency (HF; D) band power of the PI spectrum. (E,F) LF (E) and HF (F) bands in normalized units (n.u.) of the PI spectrum. (G) Band ratio between spectral powers of LF and HF components of the PI. (H) Representative power spectrum of one animal from each group. Bars represent means±s.e.m. of the control (n=11), RRS (n=10) and CVS (n=10) groups (*P<0.05 compared with the control group, one-way ANOVA followed by Bonferroni's *post hoc* test).

2015; Cruz et al., 2012; Duarte et al., 2015a; Crestani, 2016). Most results are contradictory and this may be related to the age difference, rat strain, type of stress and intensity of aversive stimulus. In a study by De Boer et al. (1989) it was shown that regular, but not irregularly, applied noise increased plasma noradrenaline concentration, while noradrenaline response to noise decreased in animals subjected to irregular protocols (De Boer et al., 1989). In contrast, it has been shown that although repeated exposure to restraint stress indicates a decrease in pressure and the tachycardic response (Bechtold et al., 2009), other studies have reported similar cardiovascular responses during both acute and repeated chronic exposure (Conti et al., 2001; Daubert et al., 2012), suggesting that there may not be a cardiovascular habituation. Thus, it is possible that RRS promotes a higher cardiovascular demand, which leads to baroreflex impairment due to increased BP and f_H during daily stress exposure, which does not

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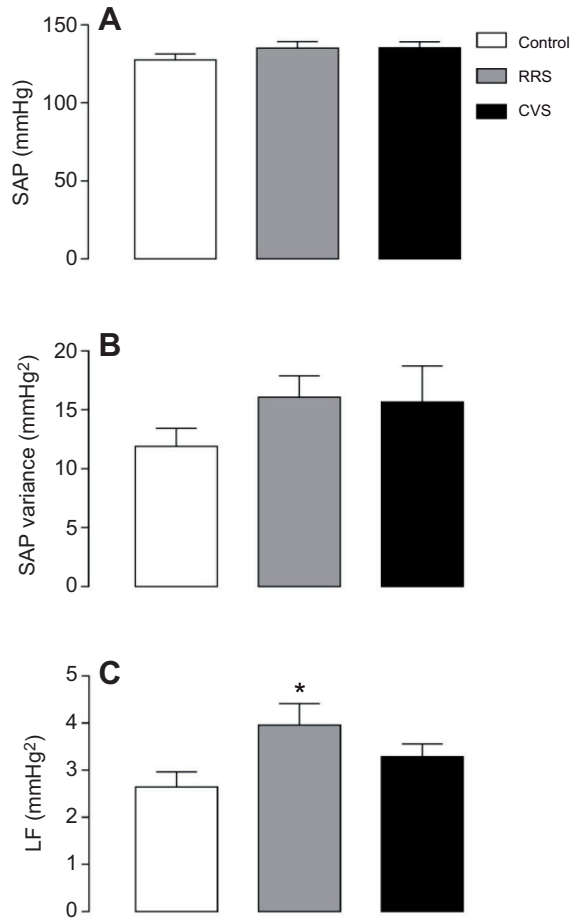

Fig. 5. Effects of chronic stress on blood pressure. (A) Systolic arterial pressure (SAP). (B) Variability of SAP. (C) LF band of the SAP spectrum. The bars represent means±s.e.m. of control (n=11), RRS (n=10) and CVS (n=10) groups (*P<0.05 versus control group, one-way ANOVA followed by Bonferroni's *post hoc* test).

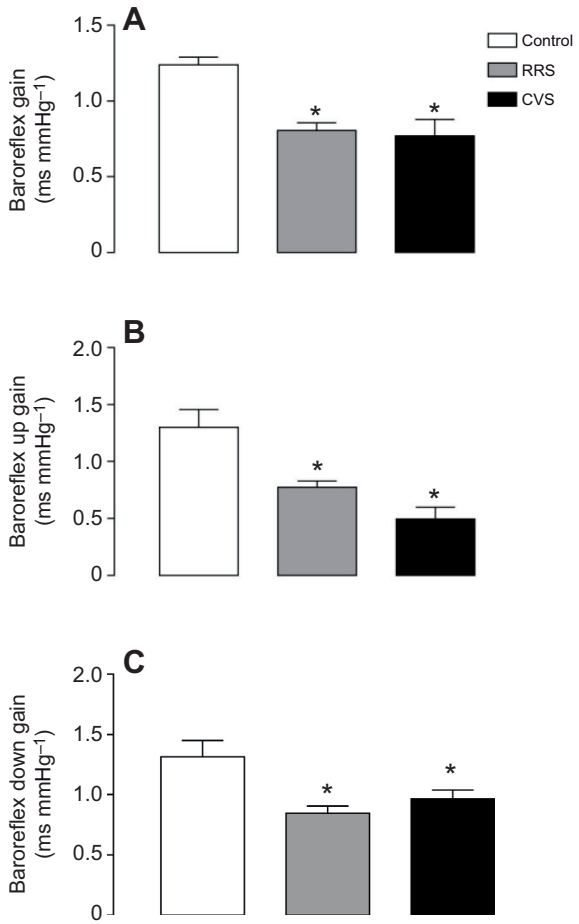

Fig. 6. Effects of chronic stress on spontaneous baroreflex sensitivity. (A) Gain of all baroreflex sequences, (B) baroreflex (SAP) increases associated with PI lengthening (up gain) and (C) baroreflex (SAP) decreases associated with PI shortening (down gain) for animals subjected to chronic stress protocols. The bars represent means±s.e.m. of control (n=11), RRS (n=10) and CVS (n=10) groups (*P<0.05 versus control group, one-way ANOVA followed by Bonferroni's *post hoc* test).

happen in the CVS protocol. Reports in the literature demonstrate that exacerbated tachycardic responses constitute an important risk factor for myocardial ischemia and sudden death (Palatini and Julius, 1997). Thus, the potentiation of tachycardic reflex responses after RRS may be a risk factor for cardiovascular disease. However, facilitation of bradycardic responses may serve as a mechanism for attenuation of cardiac changes induced by chronic stress (Duarte et al., 2015a), which may contribute to the lack of effect on the basal levels of MAP and f_H.

It is well established that the baroreceptor reflex is a homeostatic response modulated by the autonomic nervous system. In mammals, baroreceptors are present in nerve endings found in the carotid sinus and aortic arch (Papademetriou et al., 2011). However, structures of the limbic system and forebrain have been shown to connect to brainstem regions responsible for modulation in autonomic responses (Berntson and Cacioppo, 2007).

In this context, studies have shown that the stimulation or inhibition of these limbic structures, such as the medial prefrontal cortex (Resstel et al., 2004; Resstel et al., 2006; Lagatta et al., 2015), anterior hypothalamus (Simon et al., 1985) or posterior hypothalamus (Bauer et al., 1988), hippocampus (Ferreira-Junior et al., 2017) and periaqueductal gray matter (Pelosi et al., 2007), is able to influence the function of the baroreflex. Similarly, chronic stress causes structural remodeling in neurons in both the hippocampus and the prefrontal cortex (Cook and Wellman, 2004; Radley et al., 2004; McEwen et al., 2016). Thus, changes observed in the baroreflex response may reflect the alterations induced by chronic stress in these structures.

Stressors are often associated with an increase in sympathetic cardiac control, or a decrease in parasympathetic activity, or both (Berntson and Cacioppo, 2007). On account of this, there is an increasing interest in the study of heart rate variability among researchers from diverse fields. Several studies have investigated the association between stress and the decrease of heart rate variability (HRV) (Clays et al., 2011; Kang et al., 2004; Kim et al., 2018; Duarte et al., 2015b). It is noteworthy to mention that the analysis of f_H and BP fluctuations reflects the ability of the regulatory mechanisms to respond to a wide variety of scenarios. It is well known that the higher the HRV, the better is the capacity to adjust f_H level accordingly to internal and/or external stimuli. However, the lower the BP variability, the better are the regulatory mechanisms that monitor BP oscillations on a beat-to-beat basis (Kim et al., 2018). In our study, chronic stress promoted alterations in sensitivity of the spontaneous baroreflex and HRV. RRS animals showed an increase in the LF band of the SAP spectrum. LF oscillations of both SAP and PI are associated with cardiac sympathetic modulation

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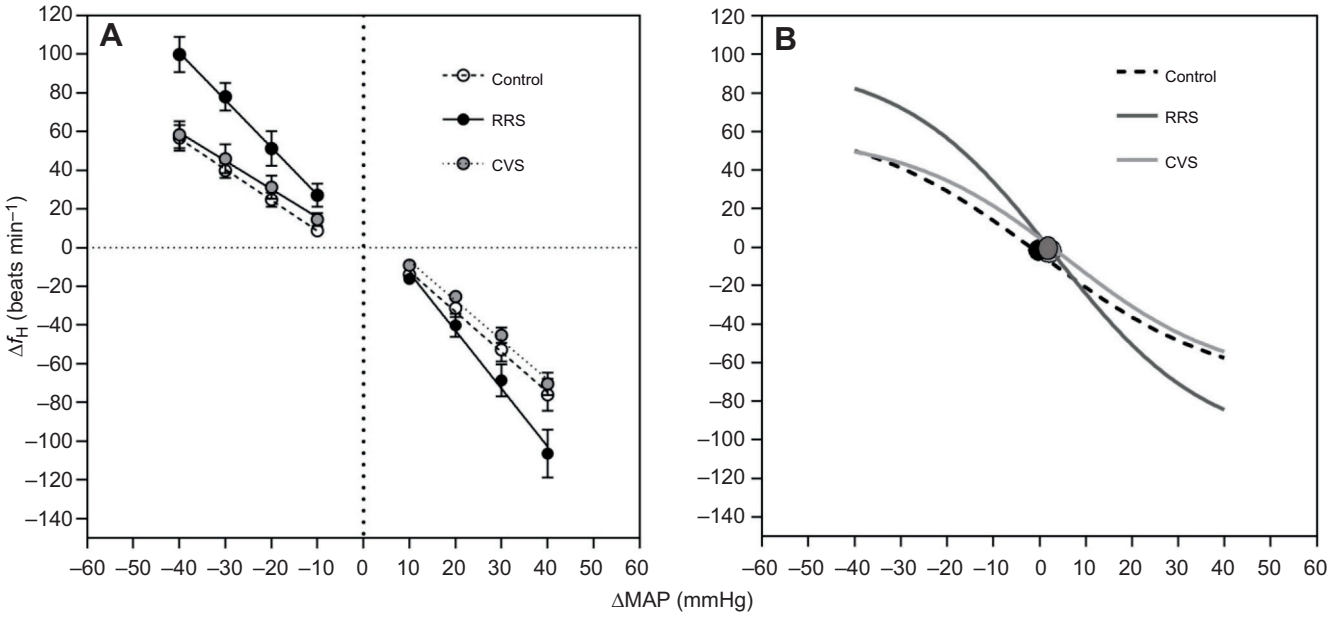

Fig. 7. Effects of chronic stress on baroreflex activity. (A) Linear regression curves correlating the variation in mean arterial pressure (ΔMAP) and heart rate (Δf_H) responses of control (n=9), RRS (n=7) and CVS (n=12) groups. Correlation r^2 values for the bradycardic regression curves were 0.66, 0.73 and 0.74, respectively. Correlation r^2 values for the tachycardic regression curves were 0.67, 0.66 and 0.38, respectively. (B) Sigmoid curves correlating ΔMAP and Δf_H of the control (r^2=0.80), RRS (r^2=0.81) and CVS (r^2=0.71) groups. Values are means±s.e.m. The circles in the sigmoidal curves represent the BP_{50} (median blood pressure).

(Malik and Camm, 1995). Despite the enhancement in the LF band of the SAP spectrum, it was not enough to modify BP. There were no changes in PI, but the chronic stress protocols decreased the potency of the LF and HF bands, as well as the power ratio (LF/HF) of these bands. The LF oscillations are mediated, essentially, by the sympathetic nervous system, and depend on the integrity of the baroreflex (Malik and Camm, 1995). In contrast, HF oscillations are related to the parasympathetic nervous activity (Malik and Camm, 1995). Despite sympathetic and parasympathetic potentiation of baroreflex activation with vasoactive drug infusion, the LF (sympathetic) and HF (parasympathetic) frequency bands of the PI spectrum were decreased.

Several studies in the literature report that chronic stress promotes increased sympathetic basal activity and alters baroreflex sensitivity (Grassi et al., 2004; Malliani et al., 1991; Duarte et al., 2015b). Recent work by Duarte et al. (2015b) demonstrated that chronic stress in adolescent animals increases the power of the LF band, the HF band and the LF/HF ratio of the PI, indicating an enhancement in both cardiac sympathetic and parasympathetic drives. In addition, regarding the sensitivity of the spontaneous baroreflex, the animals subjected to the RRS protocol presented an increase in the total baroreflex sequence gains, demonstrating an increase in the spontaneous baroreflex sensitivity. According to Grassi et al. (2004), alterations in baroreflex activity are related to hyperactivity of sympathetic tone.

Our results also show that in addition to changes in the potency of the LF and HF bands, chronic stress was also able to decrease baroreflex sensitivity analyzed by the sequence method. These results are different to the aforementioned data. Nevertheless, it is important to highlight that sympathetic hyperactivity (Mortara et al., 1994), baroreflex deficiency (Casadei et al., 1996) or low sinus node responsiveness (Malik and Camm, 1993) may reduce the LF component. This reduction in the LF band, the LF/HF ratio, in addition to the decrease in spontaneous baroreflex sensitivity, can probably be explained by a sympathetic hyperactivity, making LF oscillations less evident in the PI spectrum. For example, in the case of patients with the most severe stages of congestive heart failure (van de Borne et al., 1997), the neurohormonal excitation displays a reduction of the heart rate modulation such that only small fluctuations that are synchronous during respiratory activity are detectable (Mortara et al., 1994). In the same sense, Borchini et al. (2018) demonstrated that a high level of stress in the workplace for a period of 1 year is associated with consistent reductions of both HF and LF power and LF/HF ratio. Moreover, LF and HF values in rest periods are also reduced or absent (Borchini et al., 2018). As previously outlined, various limbic structures are involved in the autonomic system (Resstel et al., 2004, 2006; Lagatta et al., 2015; Bauer et al., 1988; Ferreira-Junior et al., 2017; Simon et al., 1985). Recently, a meta-analysis study conducted by Thayer et al. (2012) showed that several areas, including the amygdala and the medial

Table 2. Sigmoid curve parameters generated after chronic stress for the different groups

Group	Average gain (beats min^{-1} mmHg^{-1})	f_H plateau (beats min^{-1})			BP_{50} (mmHg)
		Lower	Upper	Range	
Control (n=9)	−1.311±0.0779	−76±8.360	56.78±6.557	132.8±12.77	5.407±2.953
RRS (n=7)	−2.236±0.1454*	−108±11.48*	95.57±10.02*	204.4±15.87*	0.03813±1.992
CVS (n=12)	−1.346±0.0876	−70.42±5.81	58.42±6.929	120.5±12.82	5.302±3.045
F	$F_{2,27}$=24	$F_{2,27}$=5.80	$F_{2,27}$=6.90	$F_{2,27}$=9.43	$F_{2,27}$=0.9

Values are means±s.e.m. *P<0.05 between the control, repeated chronic stress (RRS) and chronic variable stress (CVS) groups. BP_{50}, median blood pressure.

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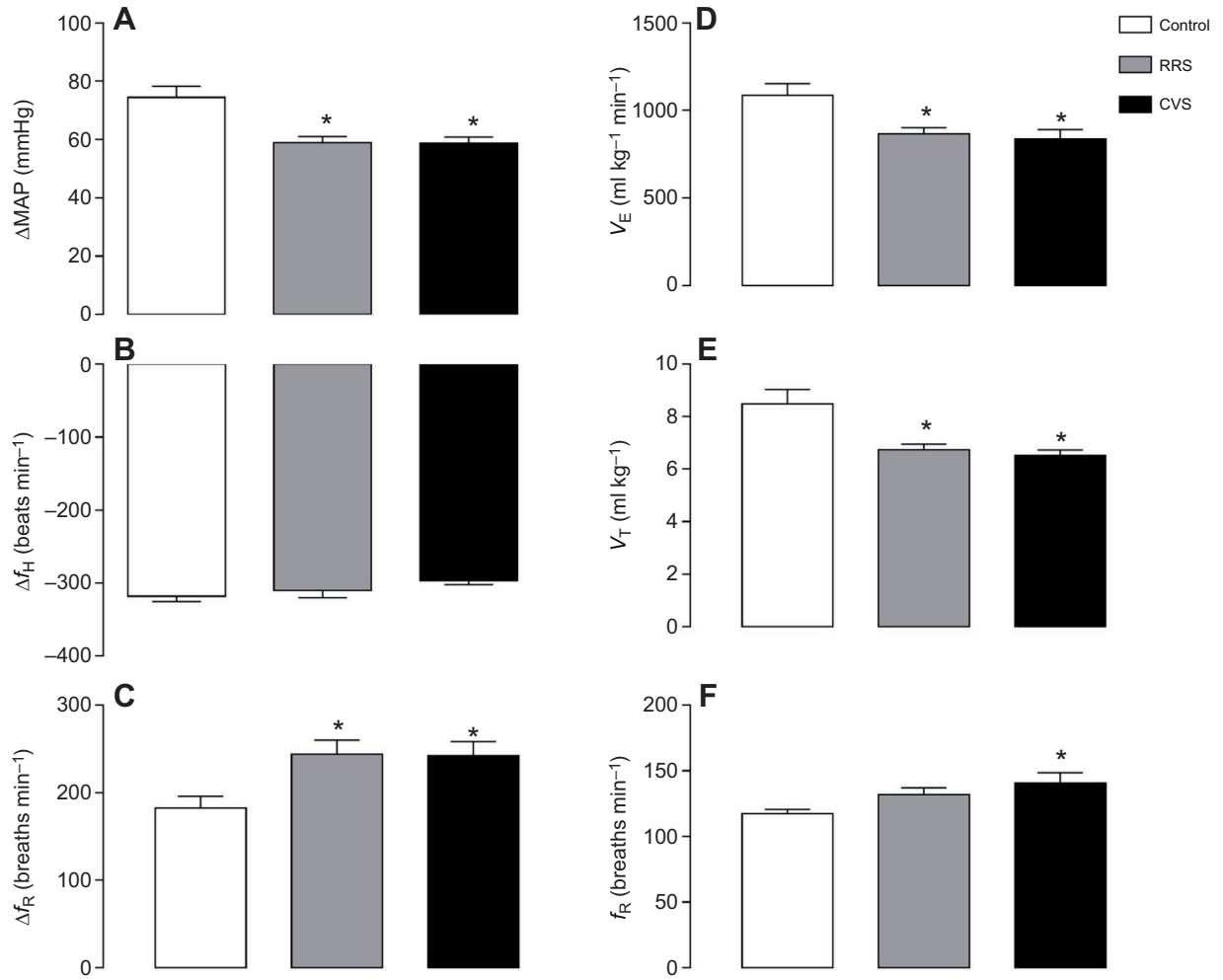

Fig. 8. Cardiovascular and respiratory responses to chemoreflex activation with KCN. (A) ΔMAP, (B) Δf_H and (C) variation in respiratory frequency (Δf_R) following chemoreflex activation with KCN (40 μg; 0.1 ml per rat i.v.). (D–F) Changes in baseline respiratory responses recorded before chemoreflex activation: (D) minute ventilation (V_E), (E) tidal volume (V_T) and (F) respiratory frequency (f_R). The bars represent means±s.e.m. of control (n=11), RRS (n=10) and CVS (n=10) groups (*P<0.05 versus control group, one-way ANOVA followed by Bonferroni's *post hoc* test).

prefrontal cortex, which are involved in the perception of threat and safety, are also associated with HRV (Thayer et al., 2012). Based on observations of stress-associated HRV and existing neurobiological evidence, HRV may be used as an objective assessment of stress and mental health. In addition, the impairment of these mechanisms by chronic stress may be the cause of the development of several cardiovascular pathologies.

Another important autonomic response that may be altered by chronic stress is the chemoreflex. This mechanism mediates cardiorespiratory responses (Fitzgerald, 2000; Paula-Ribeiro and Rocha, 2016), by maintaining arterial blood gases and acid–base balance within normal limits. However, dysfunction in the chemoreflex may impair ventilatory control and may play an important role in the pathophysiology of highly prevalent diseases, like chronic heart failure, hypertension and chronic obstructive pulmonary disease (COPD) (Paula-Ribeiro and Rocha, 2016). Our study demonstrated that RRS and CVS reduce the magnitude of the pressor response without altering the fall in f_H. This is in accordance with previous evidence supporting the concept that the sympathetic and parasympathetic components of the chemoreflex are mediated by different neural pathways and independent neurochemical mechanisms (Franchini and Krieger, 1993; Haibara et al., 1995).

Apart from their role in baroreflex control, supramedullary structures are also able to modulate cardiovascular responses activated by the chemoreflex, as inhibition of the pre-limbic cortex with cobalt chloride decreased the pressor response in awake animals without altering the bradycardic response when compared with the control group (Granjeiro et al., 2011). Additionally, the ventral hippocampus is involved in the modulation of the parasympathoexcitatory component of the peripheral chemoreflex (Kuntze et al., 2016). Chronic stress-activated regions for both RRS and CVS have been identified in the dentate gyrus, through an increase of FosB/DFosB neuronal activity marker. Additionally, CVS chronically activates the medial prefrontal cortex (Flak et al., 2012). Based on these data, chronic stress probably triggers changes in these brain areas, which in turn may impact alterations in cardiovascular responses to chemoreflex activation.

In addition to cardiovascular alterations during chemoreflex activation, chronic stress led to basal respiratory changes, such as decreased V_T and V_E and increased magnitude of f_R in response to chemoreflex activation. Additionally, both chronic stress groups

displayed an increment in the mean of the maximal response of f_R. It is known that the hypoxic ventilatory response is not the result of a single mechanism, but rather is an interaction between several different mechanisms. These mechanisms depend on specific evoking stimuli, such as intensity and exposure to hypoxia, the time course of the response (seconds to years) and the effects on ventilation components (tidal volume versus frequency) (Powell et al., 1998).

Some studies have shown that stress interferes with respiratory activity and that early stress exposure alters the developmental trajectory of the respiratory control system (Bavis and Mitchell, 2008; Cayetanot et al., 2009). According to Genest et al. (2004), early-life maternal separation induces modifications in V_E, besides altering the central and peripheral chemoreflex components of respiratory control (Kinkead et al., 2001). However, although there are reports in the literature evidencing respiratory changes caused by chronic stress in adult life, animal studies on the subject are still scarce.

In summary, the mechanisms of the baroreflex and chemoreflex play a major role in the control of the cardiovascular system via the profound influence they exert on autonomic outflow. In this way, the findings of the present study demonstrate the impact of chronic stress not only on depressive-like behavior but also on alterations of the autonomic baroreflex response and cardiocirculatory variability (SAP and PI). Additionally, our study is one of the first to show that chronic stress decreases the magnitude of the pressor response and potentiates respiratory responses to chemoreflex activation, which can trigger cardiovascular and respiratory pathologies. These alterations seem to constitute important mechanisms in the etiology of chronic stress in cardiovascular modifications. Moreover, stress is a risk factor for cardiovascular disease; however, the mechanisms linking chronic stress to increased risk of cardiac events may be partially attributed to direct or indirect effects on autonomic function. Thus, the present results provide evidence that the autonomic alterations could reflect important mechanisms underlining the etiology of cardiovascular diseases which are associated with chronic stress exposure.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: E.M.S.F.; Methodology: E.M.S.F., L.B.K., D.C.L., L.B.M.R.; Software: D.P.M.D.; Validation: E.M.S.F.; Formal analysis: E.M.S.F., L.B.K., D.C.L.; Investigation: E.M.S.F., L.B.K., D.C.L.; Resources: E.M.S.F., L.B.K., D.C.L.; Data curation: E.M.S.F.; Writing - original draft: E.M.S.F.; Writing - review & editing: E.M.S.F., L.B.K., D.C.L., D.P.M.D., L.B.M.R.; Visualization: E.M.S.F.; Supervision: L.B.M.R.; Project administration: L.B.M.R.

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References
Aguilera, G. (1998). Corticotropin releasing hormone, receptor regulation and the stress response. *Trends Endocrinol. Metab.* **9**, 329-336. doi:10.1016/S1043-2760(98)00079-4

Almeida, J., Duarte, J. O., Oliveira, L. A. and Crestani, C. C. (2015). Effects of nitric oxide synthesis inhibitor or fluoxetine treatment on depression-like state and cardiovascular changes induced by chronic variable stress in rats. *Stress* **18**, 462-474. doi:10.3109/10253890.2015.1038993

Alves, F. H., Crestani, C. C., Resstel, L. B. and Correa, F. M. (2009). N-methyl-D-aspartate receptors in the insular cortex modulate baroreflex in unanesthetized rats. *Auton. Neurosci.* **147**, 56-63. doi:10.1016/j.autneu.2008.12.015

Barrès, C., Cheng, Y. and Julien, C. (2004). Steady-state and dynamic responses of renal sympathetic nerve activity to air-jet stress in sinoaortic denervated rats. *Hypertension* **43**, 629-635. doi:10.1161/01.HYP.0000115384.01463.61

Bartlett, D. and Tenney, S. M. (1970). Control of breathing in experimental anemia. *Respir. Physiol.* **10**, 384-395. doi:10.1016/0034-5687(70)90056-3

Bauer, R. M., Vela, M. B., Simon, T. and Waldrop, T. G. (1988). A GABAergic mechanism in the posterior hypothalamus modulates baroreflex bradycardia. *Brain Res. Bull.* **20**, 633-641. doi:10.1016/0361-9230(88)90224-9

Bavis, R. W. and Mitchell, G. S. (2008). Long-term effects of the perinatal environment on respiratory control. *J. Appl. Physiol. (1985)* **104**, 1220-1229. doi:10.1152/japplphysiol.01086.2007

Bechtold, A. G., Patel, G., Hochhaus, G. and Scheuer, D. A. (2009). Chronic blockade of hindbrain glucocorticoid receptors reduces blood pressure responses to novel stress and attenuates adaptation to repeated stress. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R1445-R1454. doi:10.1152/ajpregu.00095.2008

Berntson, G. G. and Cacioppo, J. T. (2007). Heart rate variability: stress and psychiatric conditions. In *Dynamic Electrocardiography* (ed. M. Malik and A. J. Camm), pp. 57-64. Blackwell.

Borchini, R., Veronesi, G., Bonzini, M., Gianfagna, F., Dashi, O. and Ferrario, M. M. (2018). Heart rate variability frequency domain alterations among healthy nurses exposed to prolonged work stress. *Int. J. Environ. Res. Public Health* **15**, E113. doi:10.3390/ijerph15010113

Carrive, P. (2006). Dual activation of cardiac sympathetic and parasympathetic components during conditioned fear to context in the rat. *Clin. Exp. Pharmacol. Physiol.* **33**, 1251-1254. doi:10.1111/j.1440-1681.2006.04519.x

Casadei, B., Conway, J., Forfar, C. and Sleight, P. (1996). Effect of low doses of scopolamine on RR interval variability, baroreflex sensitivity, and exercise performance in patients with chronic heart failure. *Heart* **75**, 274-280. doi:10.1136/hrt.75.3.274

Cayetanot, F., Larnicol, N. and Peyronnet, J. (2009). Antenatal environmental stress and maturation of the breathing control, experimental data. *Respir. Physiol. Neurobiol.* **168**, 92-100. doi:10.1016/j.resp.2009.04.024

Clays, E., De Bacquer, D., Crasset, V., Kittel, F., De Smet, P., Kornitzer, M., Karasek, R. and De Backer, G. (2011). The perception of work stressors is related to reduced parasympathetic activity. *Int. Arch. Occup. Environ. Health* **84**, 185-191. doi:10.1007/s00420-010-0537-z

Conti, L. H., Shannon, M. H., Murry, J. D. and Printz, M. P. (2001). Repeated restraint stress-induced increase in baroreceptor reflex sensitivity: role of corticotropin-releasing factor. *Neuropeptides* **35**, 71-81. doi:10.1054/npep.2001.0847

Cook, S. C. and Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J. Neurobiol.* **60**, 236-248. doi:10.1002/neu.20025

Crestani, C. C. (2016). Emotional stress and cardiovascular complications in animal models: a review of the influence of stress type. *Front. Physiol.* **7**, 251. doi:10.3389/fphys.2016.00251

Crestani, C. C., Tavares, R. F., Alves, F. H., Resstel, L. B. and Correa, F. M. (2010). Effect of acute restraint stress on the tachycardiac and bradycardiac responses of the baroreflex in rats. *Stress* **13**, 61-72. doi:10.3109/10253890902927950

Cruz, F. C., Marin, M. T., Leão, R. M. and Planeta, C. S. (2012). Behavioral and neuroendocrine effects of the exposure to chronic restraint or variable stress in early adolescent rats. *Int. J. Dev. Neurosci.* **30**, 19-23. doi:10.1016/j.ijdevneu.2011.10.005

Curtis, A. L., Lechner, S. M., Pavcovich, L. A. and Valentino, R. J. (1997). Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J. Pharmacol. Exp. Ther.* **281**, 163-172.

Daubert, D. L., Mccowan, M., Erdos, B. and Scheuer, D. A. (2012). Nucleus of the solitary tract catecholaminergic neurons modulate the cardiovascular response to psychological stress in rats. *J. Physiol.* **590**, 4881-4895. doi:10.1113/jphysiol.2012.232314

De Boer, S. F., Van Der Gugten, J. and Slangen, J. L. (1989). Plasma catecholamine and corticosterone responses to predictable and unpredictable noise stress in rats. *Physiol. Behav.* **45**, 789-795. doi:10.1016/0031-9384(89)90296-5

Drorbaugh, J. E. and Fenn, W. O. (1955). A barometric method for measuring ventilation in newborn infants. *Pediatrics* **16**, 81-87.

Duarte, J. O., Cruz, F. C., Leão, R. M., Planeta, C. S. and Crestani, C. C. (2015a). Stress vulnerability during adolescence: comparison of chronic stressors in adolescent and adult rats. *Psychosom. Med.* **77**, 186-199. doi:10.1097/PSY.0000000000000141

Duarte, J. O., Planeta, C. S. and Crestani, C. C. (2015b). Immediate and long-term effects of psychological stress during adolescence in cardiovascular function:

comparison of homotypic vs heterotypic stress regimens. *Int. J. Dev. Neurosci.* **40**, 52-59. doi:10.1016/j.ijdevneu.2014.11.004

Engi, S. A., Cruz, F. C., Leao, R. M., Corrêa, F. M., Planeta, C. S. and Crestani, C. C. (2012). Effect of the single or combined administration of cocaine and testosterone on cardiovascular function and baroreflex activity in unanesthetized rats. *J. Cardiovasc. Pharmacol.* **59**, 231-240. doi:10.1097/FJC.0b013e31823cc58b

Esch, T., Stefano, G. B., Fricchione, G. L. and Benson, H. (2002). Stress in cardiovascular diseases. *Med. Sci. Monit.* **8**, RA93-RA101.

Fernandes, L. G., Antunes, V. R., Bonagamba, L. G. and Machado, B. H. (2005). Pressor response to chemoreflex activation in awake rats: role of vasopressin and adrenal medulla. *Physiol. Behav.* **84**, 39-44. doi:10.1016/j.physbeh.2004.10.008

Ferreira-Junior, N. C., Lagatta, D. C., Fabri, D. R., Alves, F. H., Corrêa, F. M. and Resstel, L. B. (2017). Hippocampal subareas arranged in the dorsoventral axis modulate cardiac baroreflex function in a site-dependent manner in rats. *Exp. Physiol.* **102**, 14-24. doi:10.1113/EP085827

Fitzgerald, R. S. (2000). Oxygen and carotid body chemotransduction: the cholinergic hypothesis - a brief history and new evaluation. *Respir Physiol.* **120**, 89-104. doi:10.1016/S0034-5687(00)00091-8

Flak, J. N., Solomon, M. B., Jankord, R., Krause, E. G. and Herman, J. P. (2012). Identification of chronic stress-activated regions reveals a potential recruited circuit in rat brain. *Eur. J. Neurosci.* **36**, 2547-2555. doi:10.1111/j.1460-9568.2012.08161.x

Franchini, K. G. and Krieger, E. M. (1993). Cardiovascular responses of conscious rats to carotid body chemoreceptor stimulation by intravenous KCN. *J. Auton Nerv. Syst.* **42**, 63-69. doi:10.1016/0165-1838(93)90342-R

Gadek-Michalska, A. and Bugajski, J. (2003). Repeated handling, restraint, or chronic crowding impair the hypothalamic-pituitary-adrenocortical response to acute restraint stress. *J. Physiol. Pharmacol.* **54**, 449-459.

Genest, S. E., Gulemetova, R., Laforest, S., Drolet, G. and Kinkead, R. (2004). Neonatal maternal separation and sex-specific plasticity of the hypoxic ventilatory response in awake rat. *J. Physiol.* **554**, 543-557. doi:10.1113/jphysiol.2003.052894

Granjeiro, E. M., Scopinho, A. A., Corrêa, F. M. and Resstel, L. B. (2011). Prelimbic but not infralimbic cortex is involved in the pressor response to chemoreflex activation in awake rats. *Exp. Physiol.* **96**, 518-527. doi:10.1113/expphysiol.2011.057596

Grassi, G., Seravalle, G., Dell'oro, R., Facchini, A., Ilardo, V. and Mancia, G. (2004). Sympathetic and baroreflex function in hypertensive or heart failure patients with ventricular arrhythmias. *J. Hypertens.* **22**, 1747-1753. doi:10.1097/00004872-200409000-00019

Grippo, A. J., Moffitt, J. A. and Johnson, A. K. (2002). Cardiovascular alterations and autonomic imbalance in an experimental model of depression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R1333-R1341. doi:10.1152/ajpregu.00614.2001

Grippo, A. J., Beltz, T. G. and Johnson, A. K. (2003). Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol. Behav.* **78**, 703-710. doi:10.1016/S0031-9384(03)00050-7

Grippo, A. J., Beltz, T. G., Weiss, R. M. and Johnson, A. K. (2006). The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. *Biol. Psychiatry* **59**, 309-316. doi:10.1016/j.biopsych.2005.07.010

Grippo, A. J., Moffitt, J. A. and Johnson, A. K. (2008). Evaluation of baroreceptor reflex function in the chronic mild stress rodent model of depression. *Psychosom. Med.* **70**, 435-443. doi:10.1097/PSY.0b013e31816ff7dd

Gulemetova, R., Drolet, G. and Kinkead, R. (2013). Neonatal stress augments the hypoxic chemoreflex of adult male rats by increasing AMPA receptor-mediated modulation. *Exp. Physiol.* **98**, 1312-1324. doi:10.1113/expphysiol.2013.072090

Haibara, A. S., Colombari, E., Chianca, D. A., , Jr, Bonagamba, L. G. and Machado, B. H. (1995). NMDA receptors in NTS are involved in bradycardic but not in pressor response of chemoreflex. *Am. J. Physiol.* **269**, H1421-H1427. doi:10.1152/ajpheart.1995.269.4.H1421

Haibara, A. S., Bonagamba, L. G. and Machado, B. H. (1999). Sympathoexcitatory neurotransmission of the chemoreflex in the NTS of awake rats. *Am. J. Physiol.* **276**, R69-R80. doi:10.1152/ajpregu.1999.276.1.R69

Inoue, N. (2014). Stress and atherosclerotic cardiovascular disease. *J. Atheroscler. Thromb.* **21**, 391-401. doi:10.5551/jat.21709

Irvine, R. J., White, J. and Chan, R. (1997). The influence of restraint on blood pressure in the rat. *J. Pharmacol. Toxicol. Methods* **38**, 157-162. doi:10.1016/S1056-8719(97)00081-6

Kang, M. G., Koh, S. B., Cha, B. S., Park, J. K., Woo, J. M. and Chang, S. J. (2004). Association between job stress on heart rate variability and metabolic syndrome in shipyard male workers. *Yonsei Med. J.* **45**, 838-846. doi:10.3349/ymj.2004.45.5.838

Kim, H.-G., Cheon, E.-J., Bai, D.-S., Lee, Y. H. and Koo, B.-H. (2018). Stress and heart rate variability: a meta-analysis and review of the literature. *Psychiatry Investig.* **15**, 235-245. doi:10.30773/pi.2017.08.17

Kinkead, R., Bach, K. B., Johnson, S. M., Hodgeman, B. A. and Mitchell, G. S. (2001). Plasticity in respiratory motor control: intermittent hypoxia and hypercapnia activate opposing serotonergic and noradrenergic modulatory systems. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **130**, 207-218. doi:10.1016/S1095-6433(01)00393-2

Kolber, B. J., Wieczorek, L. and Muglia, L. J. (2008). Hypothalamic-pituitary-adrenal axis dysregulation and behavioral analysis of mouse mutants with altered glucocorticoid or mineralocorticoid receptor function. *Stress* **11**, 321-338. doi:10.1080/10253890701821081

Kuntze, L. B., Ferreira-Junior, N. C., Lagatta, D. C. and Resstel, L. B. (2016). Ventral hippocampus modulates bradycardic response to peripheral chemoreflex activation in awake rats. *Exp. Physiol.* **101**, 482-493. doi:10.1113/EP085393

Lagatta, D. C., Ferreira-Junior, N. C. and Resstel, L. B. (2015). Medial prefrontal cortex TRPV1 channels modulate the baroreflex cardiac activity in rats. *Br. J. Pharmacol.* **172**, 5377-5389. doi:10.1111/bph.13327

Levine, S. (2005). Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* **30**, 939-946. doi:10.1016/j.psyneuen.2005.03.013

Lupien, S. J. and Mcewen, B. S. (1997). The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Res. Brain Res. Rev.* **24**, 1-27. doi:10.1016/S0165-0173(97)00004-0

Magariños, A. M. and McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. *Neuroscience* **69**, 83-88. doi:10.1016/0306-4522(95)00256-I

Malik, M. and Camm, A. J. (1993). Components of heart rate variability–what they really mean and what we really measure. *Am. J. Cardiol.* **72**, 821-822. doi:10.1016/0002-9149(93)91070-X

Malik, M. and Camm, A. J. (1995). *Heart Rate Variability*, Armonk, NY, Futura Pub. Co.

Malliani, A., Pagani, M., Lombardi, F. and Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation* **84**, 482-492. doi:10.1161/01.CIR.84.2.482

Marin, M. T., Cruz, F. C. and Planeta, C. S. (2007). Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiol. Behav.* **90**, 29-35. doi:10.1016/j.physbeh.2006.08.021

Marques, A. H., Silverman, M. N. and Sternberg, E. M. (2010). Evaluation of stress systems by applying noninvasive methodologies: measurements of neuroimmune biomarkers in the sweat, heart rate variability and salivary cortisol. *Neuroimmunomodulation* **17**, 205-208. doi:10.1159/000258725

Martí, O., Martí, J. and Armario, A. (1994). Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol. Behav.* **55**, 747-753. doi:10.1016/0031-9384(94)90055-8

McEwen, B. S. (2000). The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* **886**, 172-189. doi:10.1016/S0006-8993(00)02950-4

McEwen, B. S., Eiland, L., Hunter, R. G. and Miller, M. M. (2012). Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology* **62**, 3-12. doi:10.1016/j.neuropharm.2011.07.014

Mcewen, B. S., Nasca, C. and Gray, J. D. (2016). Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. *Neuropsychopharmacology* **41**, 3-23. doi:10.1038/npp.2015.171

Mortara, A., La Rovere, M. T., Signorini, M. G., Pantaleo, P., Pinna, G., Martinelli, L., Ceconi, C., Cerutti, S. and Tavazzi, L. (1994). Can power spectral analysis of heart rate variability identify a high risk subgroup of congestive heart failure patients with excessive sympathetic activation? A pilot study before and after heart transplantation. *Br Heart J.* **71**, 422-430. doi:10.1136/hrt.71.5.422

Myers, B., McKlveen, J. M. and Herman, J. P. (2014). Glucocorticoid actions on synapses, circuits, and behavior: implications for the energetics of stress. *Front. Neuroendocrinol.* **35**, 180-196. doi:10.1016/j.yfrne.2013.12.003

Nakata, T., Berard, W., Kogosov, E. and Alexander, N. (1993). Cardiovascular change and hypothalamic norepinephrine release in response to environmental stress. *Am. J. Physiol.* **264**, R784-R789. doi:10.1152/ajpregu.1993.264.4.R784

Olivan, M. V., Bonagamba, L. G. H. and Machado, B. H. (2001). Involvement of the paraventricular nucleus of the hypothalamus in the pressor response to chemoreflex activation in awake rats. *Brain Res.* **895**, 167-172. doi:10.1016/S0006-8993(01)02067-4

Ortiz, J., Fitzgerald, L. W., Lane, S., Terwilliger, R. and Nestler, E. J. (1996). Biochemical adaptations in the mesolimbic dopamine system in response to repeated stress. *Neuropsychopharmacology* **14**, 443-452. doi:10.1016/0893-133X(95)00152-4

Palatini, P. and Julius, S. (1997). Association of tachycardia with morbidity and mortality: pathophysiological considerations. *J. Hum. Hypertens.* **11** Suppl. 1, S19-S27.

Papademetriou, V., Doumas, M., Faselis, C., Tsioufis, C., Douma, S., Gkaliagkousi, E. and Zamboulis, C. (2011). Carotid baroreceptor stimulation for the treatment of resistant hypertension. *Int. J. Hypertens* **2011**, 964394. doi:10.4061/2011/964394

Paula-Ribeiro, M. and Rocha, A. (2016). The peripheral-central chemoreflex interaction: where do we stand and what is the next step? *J. Physiol.* **594**, 1527-1528. doi:10.1113/JP271901

Pelosi, G. G., Resstel, L. B. and Corrêa, F. M. (2007). Dorsal periaqueductal gray area synapses modulate baroreflex in unanesthetized rats. *Auton. Neurosci.* **131**, 70-76. doi:10.1016/j.autneu.2006.07.002

Porges, S. W. (1995). Cardiac vagal tone: a physiological index of stress. *Neurosci. Biobehav. Rev.* **19**, 225-233. doi:10.1016/0149-7634(94)00066-A

Powell, F. L., Milsom, W. K. and Mitchell, G. S. (1998). Time domains of the hypoxic ventilatory response. *Respir Physiol.* **112**, 123-134. doi:10.1016/S0034-5687(98)00026-7

Radley, J. J., Sisti, H. M., Hao, J., Rocher, A. B., McCall, T., Hof, P. R., McEwen, B. S. and Morrison, J. H. (2004). Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience* **125**, 1-6. doi:10.1016/j.neuroscience.2004.01.006

Resstel, L. B. M., Fernandes, K. B. P. and Corrêa, F. M. A. (2004). Medial prefrontal cortex modulation of the baroreflex parasympathetic component in the rat. *Brain Res.* **1015**, 136-144. doi:10.1016/j.brainres.2004.04.065

Resstel, L. B. M., Joca, S. R. L., Guimarães, F. G. and Corrêa, F. M. A. (2006). Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. *Neuroscience* **143**, 377-385. doi:10.1016/j.neuroscience.2006.08.002

Scharf, S. H. and Schmidt, M. V. (2012). Animal models of stress vulnerability and resilience in translational research. *Curr. Psychiatry Rep.* **14**, 159-165. doi:10.1007/s11920-012-0256-0

Simas, B. B., Nunes, E. A., Crestani, C. C. and Speretta, G. F. (2018). Cardiovascular and metabolic consequences of the association between chronic stress and high-fat diet in rats. *Stress* **21**, 247-256. doi:10.1080/10253890.2018.1437413

Simon, O. R., Basuray, B. N., West, W. L. and Copeland, R. (1985). Interaction between the baroreflex and anterior hypothalamic stimulation. Demonstration of a noradrenergic involvement. *Neuropharmacology* **24**, 665-675. doi:10.1016/0028-3908(85)90110-8

Tavares, R. F. and Correa, F. M. (2006). Role of the medial prefrontal cortex in cardiovascular responses to acute restraint in rats. *Neuroscience* **143**, 231-240. doi:10.1016/j.neuroscience.2006.07.030

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. and 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. doi:10.1016/j.neubiorev.2011.11.009

Ulrich-Lai, Y. M. and Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* **10**, 397-409. doi:10.1038/nrn2647

Van De Borne, P., Montano, N., Pagani, M., Oren, R. and Somers, V. K. (1997). Absence of low-frequency variability of sympathetic nerve activity in severe heart failure. *Circulation* **95**, 1449-1454. doi:10.1161/01.CIR.95.6.1449

Vieira, J. O., Duarte, J. O., Costa-Ferreira, W., Morais-Silva, G., Marin, M. T. and Crestani, C. C. (2018). Sex differences in cardiovascular, neuroendocrine and behavioral changes evoked by chronic stressors in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **81**, 426-437. doi:10.1016/j.pnpbp.2017.08.014

Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl.)* **134**, 319-329. doi:10.1007/s002130050456

Willner, P. (2005). Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* **52**, 90-110. doi:10.1159/000087097

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P. and Sahebkar, A. (2017). The impact of stress on body function: a review. *EXCLI J.* **16**, 1057-1072.