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Symbolic analysis of heart rate fluctuations identifies cardiac autonomic modifications during LPS-induced endotoxemia

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ABSTRACT

The present study aimed to compare linear and symbolic dynamics (SD) indices for detecting the autonomic cardiac changes produced by endotoxemia in freely-moving rats. In this context, we analyzed ECG-derived R-R time series in freely moving Dark Agouti rats, which received lipopolysaccharide (LPS, $n = 9$), or vehicle (V, $n = 7$). Five minutes R-R time series were assessed every hour up to +12 h and + 24 h post-LPS injection.

We found that SD indices showed significant differences at +7 h between V vs. LPS groups and at +9 h between basal levels of LPS (−3 h) and post-LPS injection (pre-LPS vs. post-LPS). In general, SD seems more appropriate than linear indices to evaluate the autonomic changes of endotoxemic rats. Overall, the symbolic parameters detected decreased R-R variability and complexity, which indicate a modification of the autonomic regulation during LPS-induced endotoxemia. This modification is probably related to a reduced activity of the cholinergic anti-inflammatory pathway at the long term.

1. Introduction

The autonomic nervous system (ANS) and the inflammatory response are functionally linked. The acute response to an inflammatory insult includes the activation of innate immune mechanisms as well as changes in the autonomic nervous activity (Tracey, 2002). Sympathetic and vagal nerves are documented to produce anti-inflammatory effects (Rosas-Ballina et al., 2008). Specifically, the efferent vagal activity influences the immune function through a powerful neural reflex that suppresses the release of critical pro-inflammatory cytokines after an immune challenge; this neuro-immune mechanism is referred to as the cholinergic anti-inflammatory reflex (Martelli et al., 2014).

It is known that systemic inflammatory scenarios, such as lipopolysaccharide (LPS)-induced endotoxemia, produce autonomic modifications that reflect sympathetic augmentation and parasympathetic attenuation (Foteinou et al., 2011). The autonomic cardiac regulation is generally assessed by the linear analysis of heart rate variability (HRV) (Ziegler et al., 2015; Zila et al., 2016). However, linear measures of HRV are not always suitable to quantify the complex dynamics of physiological systems and their corresponding time series (Fernandes de Godoy, 2016).

At present, it seems that only few studies have introduced nonlinear metrics to investigate the cardiac autonomic changes during systemic inflammatory scenarios: LPS-induced endotoxemia (Reyes-Lagos et al., 2016), human parturition (Reyes-Lagos et al., 2015) and diabetes (Meamar et al., 2015). Mathematical models of human endotoxemia indicate that an increased heart rate, as well as an increased sympathetic and/or decreased parasympathetic activities, could all be related with an uncoupling between the autonomic nervous system and the heart (Scheff et al., 2014). Other findings suggest that both sympathetic and vagal autonomic branches are enhanced following endotoxemia. However, the overexcited sympathetic system seems to lead to a sympathetic-vagal disequilibrium (Huang et al., 2010). Additionally, some authors have demonstrated that the sympathetic activation is also

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capable of producing an anti-inflammatory response in peripheral local processes of inflammation such as joint inflammation in experimental arthritis (Bassi et al., 2015).

Elsewhere, another nonlinear approach as applied to HRV data, known as symbolic dynamics (SD), has been useful to assess the activity of the cardiac autonomic nervous system (Cysarz et al., 2013; Kurths et al., 1995; Voss et al., 1996). Recent studies exhibited that some indices provided by SD are highly correlated with the cardiac autonomic modulation, and they even offer evidence for the superiority of symbolic over linear analysis (Silva et al., 2017a). Despite the SD analysis capacity to detect autonomic modifications, no studies have been conducted to evaluate the autonomic changes due to endotoxemia.

Within this framework, the present study aimed to compare linear and SD measurements for assessing the short-, intermediate- and long-term autonomic cardiac changes produced by LPS-endotoxemia in freely moving rats. We hypothesized that SD should reflect both a decreased variability and complexity of heart rate fluctuations resulting from the above suggested sympathetic-vagal disequilibrium during endotoxemia.

2. Materials and methods

2.1. R-R interval time series selection

ECG-derived R-R interval time series reported in previous studies (Reyes-Lagos et al., 2016) were extracted from our database to apply linear and SD measurements of HRV. Briefly, ECG recordings sampled at 2000 Hz were captured from 16 adult male Dark Agouti rats (DA/HanRj, 230–250 g) implanted with a telemetry transmitter as described previously (Reyes-Lagos et al., 2016). Animals were maintained on a reversed 12:12 h light/dark cycle (lights off at 7:00 AM) and had *ad libitum* access to water and chow standard diet. The recordings were divided into two different treatment groups: a) vehicle (V; saline solution, $n = 7$) and b) lipopolysaccharide (LPS; 0.1 mg/kg administered intraperitoneally, $n = 9$). Baseline recordings started at 7:00 AM (time = −3 h or reference time) and drug administration was performed at 10:00 AM (time = 0 h) in each group. Five minutes of continuous ECG-derived R-R interval time series were manually selected systematically at the beginning of each hour (from −3 to +12 h) and after 24 h. Noteworthy, this manual selection of electrocardiograms was necessary to avoid loss of data introduced by rodent's movements (motion artefact) and to assure obtaining continuous time series with no interruptions.

All experimental procedures were performed at the animal's facility of the University Hospital Essen and followed the Animal Welfare Act (TierSchG) − Germany, the European Directive 2010/63/EU, and the National Institutes of Health – the USA Animal Care guidelines. All procedures were approved by the Institutional Animal Care and Use Committee (LANUV Düsseldorf, NorthRhine-Westphalia, Germany).

2.2. HRV short-term analysis

In this study, a total of 18 HRV indices were obtained by applying both linear and SD HRV analysis on filtered R-R time series (> Wessel et al., 2007). All HRV analyses, explained in greater detail in Voss et al., 1996 and Malik et al., 1996., are briefly described as it follows.

2.2.1. Linear indices

From the time and frequency domains, the following HRV indices were calculated as complementary metrics of our previous studies (Elorza-Ávila et al., 2017; Reyes-Lagos et al., 2016):

SDANN1 [ms]: standard deviation of the 1-min average of NN (R-R) intervals.

PNN3 [%]: percentage derived by dividing the actual number of consecutive NN intervals differences > 3 ms and the total number of NN intervals.

PNN6 [%]: percentage obtained by dividing the actual number of consecutive NN interval differences > 6 ms and the total number of NN intervals.

PNN9 [%]: percentage derived by dividing the actual number of consecutive NN interval differences > 9 ms and the total number of NN intervals.

HF [ms^2]: spectral power in the high-frequency band 1.0–2.0 Hz (Barbosa-Neto et al., 2017).

LF/HF: ratio between the low- and high-frequency spectral powers.

2.2.2. Symbolic dynamics (SD)

2.2.2.1. First symbolization: σ-method. A SD representation of R-R time series using four symbols was introduced by Wessel et al. (Wessel et al., 2000) and is given by the equation:

$$S_i(x_i)\begin{cases} 0: & \mu & <xi & \leq & (1+a)\mu \\ 1: & (1+a)\mu & <x_i & < & \infty \\ 2: & (1-a)\mu & <x_i & \leq & \mu \\ 3: & 0 & <xi & \leq & (1-a)\mu \end{cases} \quad (1)$$

In Eq. (1), the transformation into symbols $S_i(x_i)$ is obtained using four non-uniform quantization levels, where μ represents the mean beat-to-beat R-R interval and, a is a setting parameter that we selected equal to 0.05 following previous studies (Cysarz et al., 2013). There are several quantities that describe or characterize the resulting strings of symbols. In this study, we analyzed the frequency distribution of words of length W = 3, i.e., substrings which consist of three successive symbols from the alphabet A = {0,1,2,3}, leading to maximal $4^W = 64$ different words.

Symbols '0' and '2' reflect low deviations (a decrease or increase) from the mean RR interval, whereas '1' and '3' reflect stronger deviations (a decrease or increase over a predefined limit). High percentages of words consisting only of the symbols '0' and '2' (WPSUM02) reflect decreased HRV. On the other hand, an indicator of increased HRV (WPSUM13) consists of a higher percentage of all words containing the symbols '1'and '3' (Voss et al., 1996).

Another measure of SD is the parameter WSDVAR, which quantifies the variability of the time series depending on a word sequence (Voss et al., 2000, 1996). Based on symbolic representations and their probabilities of occurrence, it is also possible to calculate the Shannon entropy (FWSHANNON) from the probabilities p_k of such words as follows:

$$H(W) = -\sum_{k=1}^{4^W} p_k \log p_k \quad (2)$$

A generalization of Shannon entropy is the Renyi entropy (FWRENYI); larger values of the Shannon and Renyi entropies disclose high complexity in the corresponding R-R time series. The following expression defines the FWRENYI:

$$H(W) = \frac{1}{1-\alpha}\log\left(\sum_{k=1}^{4^W} {p_k}^{\alpha}\right) \quad (3)$$

where α is a real number greater than zero. The α value can be adjusted to weigh probabilities differently. In our case, we have considered these two possibilities using $\alpha = 0.25$ (FWRENYI0.25) and $\alpha = 4$ (FWRENYI4.0).

Another parameter that may be computed from the probability distribution of words is the number of forbidden words (FORBWORD) of length 3, i.e., the number of words that never or seldom occur. A larger number of forbidden words is associated with more regularity in the R-R time series (Voss et al., 1996).

2.2.2.2. Second symbolization: binary Δ-coding-method. Wessel et al. presented a symbolic description with two symbols using the symbols

"0" and "1" to indicate differences of R-R intervals below or above a threshold (Wessel et al., 2000), i.e.:

$$S_n(x_n)\begin{cases}0: |RR_n - RR_{n-1}| < \Delta ms\\ 1: |RR_n - RR_{n-1}| \geq \Delta ms\end{cases} \quad (4)$$

where Δms indicates the time difference threshold. A measure based on this kind of symbolic sequences is the probability of low variability (POLVARΔ) that equals the probability of occurrence of the subsequence of six zeros "000000" in the symbolic string. In our comparison of features, we explored different Δ (*from* 1 *to* 6 *ms*), i.e., POLVAR1, POLVAR2, POLVAR3, POLVAR4, POLVAR5, and POLVAR6, respectively. For instance, POLVAR2 is the probability of occurrence of the subsequence "000000" with a Δ of 2 ms in the Eq. (4). Another measure is the probability of high variability, or PHVARΔ, which is equal to the probability of occurrence of six ones "111111" in the symbolic string (Voss et al., 2013).

2.3. Statistical analysis

The results are presented as mean ± standard error of the mean (SEM). Two-way ANOVA with repeated measures was used to assess the effect of LPS-endotoxemia over time after drug injection on linear HRV and SD indices. Data were further analyzed by Bonferroni post hoc test and corrected for multiple comparisons. $\alpha = 0.05$ was considered as the significance level. GraphPad software (Prism 7.0) was used to assess the statistical analyses.

3. Results

Endotoxin (LPS, 1 mg/kg) effects on 5 min of R-R or inter-beat intervals extracted at the beginning of each hour are shown for the linear indices of HRV (Fig. 1), for the SD parameters of the first symbolization or σ-method (Fig. 2), and for the second symbolization or binary Δ-coding-method (Fig. 3). The ECG recording lasted from 3 h (−3 h, reference time) before endotoxin injection up to +12 h and + 24 h post-LPS injection.

A significant decrement of SDANN1 was found at +7 h in the LPS group in comparison with -V- (Two-way ANOVA, $F_{\text{vehicle versus LPS}} = 1.81$, $P < 0.05$; Fig. 1a) and also at the same time compared to basal levels of SDANN1 ($F_{\text{preLPS versus postLPS}} = 2.60$, $P < 0.001$). No differences were found at any time point between V and LPS (or between pre- and post-LPS) for PNN3 (Fig. 1b), PNN6 (Fig. 1c), PNN9 (Fig. 1d) and HF (Fig. 1e). However, LF/HF exhibited a significant decrement at +4 h ($F_{\text{vehicle versus LPS}} = 1.75$, $P < 0.05$; Fig. 1f) and trending level differences at +7 h ($P = 0.053$).

A significant increment of FORBWORD accompanied the acute endotoxin challenge at +7 h in comparison with vehicle ($F_{\text{vehicle versus LPS}} = 4.68$, $P < 0.0001$; Fig. 2a). Likewise, we found significant differences in FWSHANNON between the vehicle and endotoxemic rats at +7 h ($F_{\text{vehicle versus LPS}} = 7.27$, $P < 0.001$; Fig. 2b). For FWRENYI0.25, we found significant differences at +2 h and +7 h ($F_{\text{vehicle versus LPS}} = 7.29$, $P < 0.0001$; Fig. 2c). On the other hand, FWRENYI4.0 showed few significant differences (data not shown).

The WSDVAR, WPSUM02 and WPSUM13 exhibited differences at +7 h ($F_{\text{vehicle versus LPS}} = 3.70$, $P < 0.0001$; Fig. 2d; $F_{\text{vehicle versus LPS}} = 2.40$, $P < 0.01$; Fig. 2e and $F_{\text{vehicle versus LPS}} = 2.07$, $P < 0.05$; Fig. 2f), respectively. Additionally, we found significant decrements in the LPS group at +7 and + 9 h compared to basal FWRENYI0.25 and WSDVAR values ($F_{\text{preLPS versus postLPS}} = 2.97$, $P < 0.001$; Fig. 2c and $F_{\text{preLPS versus postLPS}} = 4.27$, $P < 0.001$; Fig. 2d), respectively.

We also looked at other symbolic parameters such as POLVARΔ. In particular, the POLVAR2 (Fig. 3b) exhibited the highest number of significant differences and the lowest *p*-values in comparison with other POLVARΔ metrics. These results indicate that endotoxin increased the mean values of POLVAR2 in contrast with basal values from +9 h to +12 h ($F_{\text{preLPS versus postLPS}} = 3.39$, $P < 0.0001$; Fig. 3b). Additionally, we found that also POLVAR2 showed differences between V vs. LPS at +10 h and +12 h ($F_{\text{vehicle versus LPS}} = 3.70$, $P < 0.0001$; Fig. 3b). For comparison, we evaluated the probability of high variability (PHVAR1 and PHVAR2). However, these parameters did not show significant differences at any time (data not shown).

In Fig. 4, we show a summary of p-values between V vs. LPS at +7 h and between pre- and post-LPS (+3 h vs. +9 h) for all of the 18 HRV indices analyzed. As one can see in Fig. 4, the HRV quantified by WSDVAR showed clearer significant differences at +7 h. Additionally, POLVAR2 was the parameter with the lowest p-values at +9 h.

4. Discussion

Nonlinear methods have been introduced in previous studies to characterize HRV data, which according to some authors present the advantage of not being affected by non-stationary effects in comparison with traditional linear HRV indices (Buccelletti et al., 2012). Interestingly, other studies have already implemented additional nonlinear measures to evaluate the effect of endotoxemia in diabetic rats with promising results (Meamar et al., 2015). To our best knowledge, this is the first study documenting how an endotoxemic process alters the SD of HRV data.

It is well-known that LPS-induced endotoxemia is associated with a reduction of the autonomic modulation as a consequence of the systemic inflammation (Foteinou et al., 2011); thereby, some authors consider that endotoxemia provokes a cardiac autonomic dysregulation (Scheff et al., 2011). It is also important to take into consideration that the SD parameters quantify increased or decreased HRV and its complexity. For example, increased POLVAR10 measures have been associated with cardiac sympathetic activation before the onset of ventricular tachycardia in humans (Wessel et al., 2007). Additionally, other studies support that the decreased values of FWSHANNON and increased values of POLVAR3 are useful to characterize the inhibition of vagal tone in rats (Miyabara et al., 2017). Thus, from a physiological interpretation, both depressed HRV and reduced complexity are associated with an impairment of the autonomic nervous system (Beckers et al., 2006; Malik et al., 1996).

Despite that some relevant linear indices have been used to evaluate cardiac autonomic modifications in rodents such as PNN3 applied in rats with streptozotocin-induced diabetes (Morozova et al., 2019); PNN6 in endotoxemic mice (Fairchild et al., 2009); and PNN9 in epileptic rats (Möller et al., 2019), we found here that these indices were not sensitive to identify cardiac autonomic modifications due to moderate endotoxemia. Yet SD metrics have shown cardiac autonomic alterations of congestive heart failure rats in comparison with linear indices; thereby suggesting that the symbolic analysis provides robust measures of the autonomic modulation (Tobaldini et al., 2009). Our current results are in line with that study and our previous findings, which showed that time-domain indices of HRV exhibited differences only from +7 h to +9 h ($P < 0.01$) between endotoxemic and control rats (Reyes-Lagos et al., 2016). In the present study, the analysis of the SD indicated additional significant LPS-driven differences with lower *p*-values ($P < 0.0001$) in comparison with those obtained by linear analysis (Fig. 4). Thus, our current findings suggest that the SD analysis appears to provide more sensitive measures to detect the adjustments in cardiac autonomic regulation induced by endotoxemia.

These measures show that during LPS-induced endotoxemia at the long term the HRV presented: 1) less complexity as indicated by lower values of FWRENYI0.25 and FWSHANNON; 2) more regularity as indicated by higher FORBWORD values; 3) less variability as indicated by lower WSDVAR and WPSUM13 as well as more elevated amounts of WPSUM02 and POLVAR2. Based on these considerations, the reduced variability and decreased complexity of HRV in endotoxin-treated rats can be attributed to an autonomic dysfunction during LPS-induced endotoxemia (Foteinou et al., 2010). However, other authors have

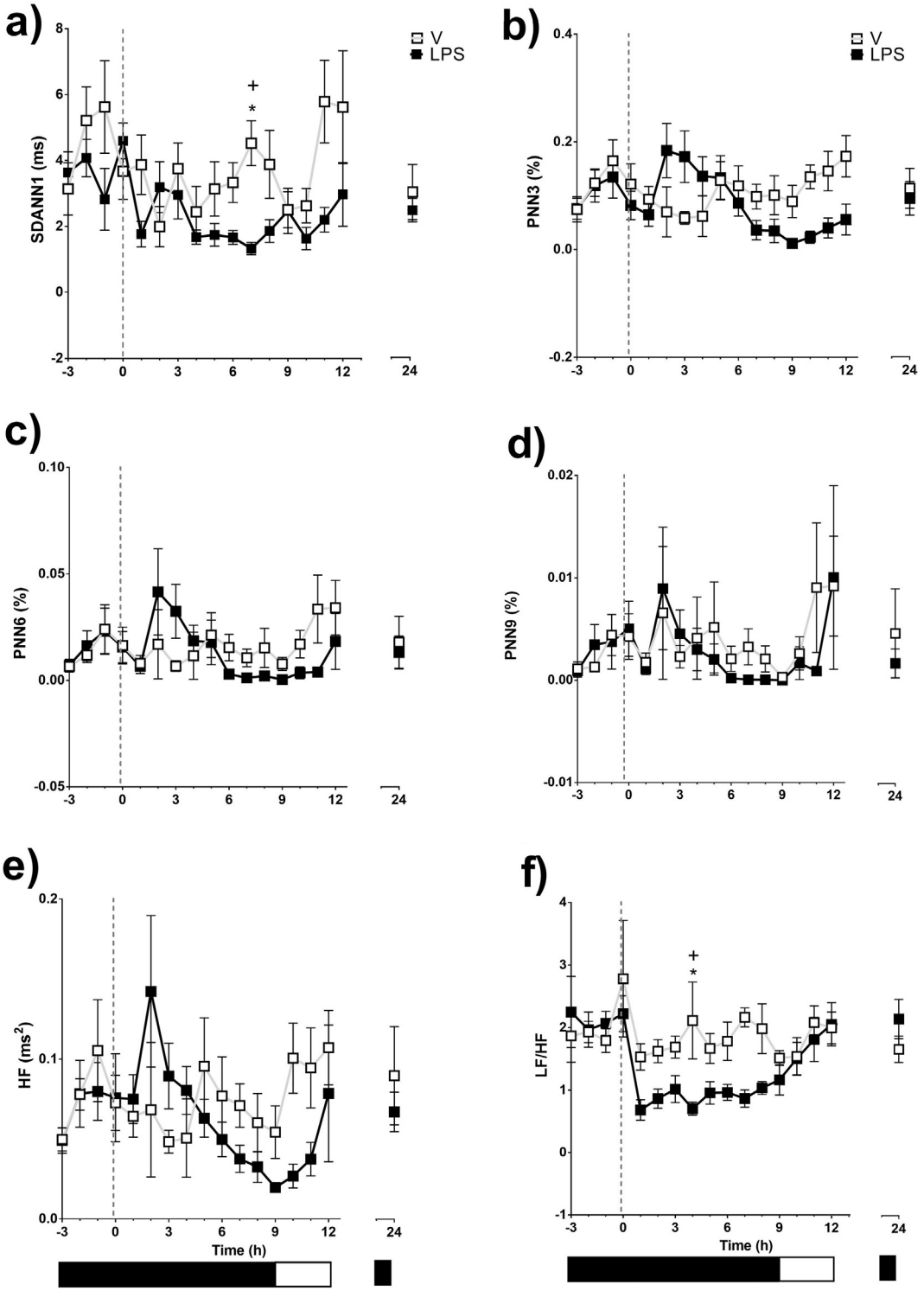

Fig. 1. Linear heart rate variability (HRV) indices of freely moving adult male Dark Agouti rats: a) standard deviation of the 1-min average of NN intervals (SDANN1); b) percentage of consecutive RR intervals that differ by > 3 ms (PNN3); c) percentage of consecutive RR intervals that differ by > 6 ms (PNN6); d) percentage of consecutive RR intervals that differ by > 9 ms (PNN9); e) spectral power in the high-frequency band (HF) and f) ratio between low- and high-frequency spectral power (LF/HF). These indices are reported before and after injection (time = 0) with vehicle (V), and lipopolysaccharide (LPS). The black/white bar at the bottom indicates the dark/light periods, respectively. Data are shown as mean ± SEM. * $p < 0.05$ LPS vs. V and + $p < 0.05$ between basal levels (−3 h) of LPS and post-LPS (pre-LPS vs. post-LPS) according to a Bonferroni posthoc test.

suggested that what it occurs during endotoxemia is a partial uncoupling of cardiac pacemaker cells from the autonomic neural control, resulting in a decreased HRV (Gholami et al., 2012). These results are in accordance with previous findings, confirming that endotoxemia at the long term is also accompanied by a significant loss of fractal heart rate dynamics, increased heart rate and decreased respiratory sinus arrhythmia (Elorza-Ávila et al., 2017; Reyes-Lagos et al., 2016). Other authors found reduced controllability in cardiac rhythm in rats following endotoxemia (Mazloom et al., 2014).

Furthermore, we previously characterized that the immunological

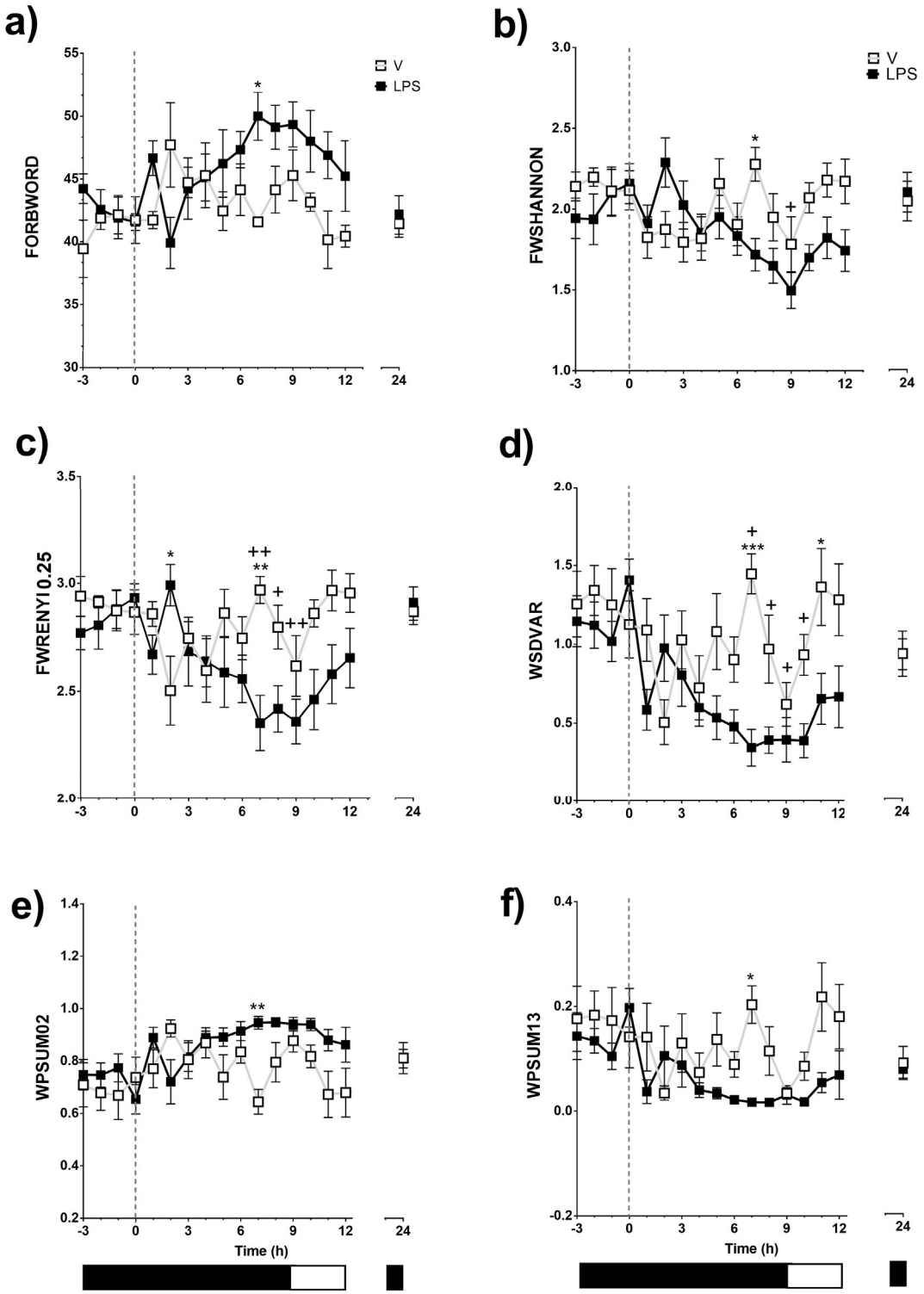

Fig. 2. First symbolization or σ-method symbolic parameters of freely moving adult male Dark Agouti rats: a) forbidden words (FORBWORD); b) Shannon entropy of the word distribution (FWSHANNON); c) Renyi entropy of the word distribution with α = 0.25 (FWRENYI0.25); d) standard deviation of the word sequence (WSDVAR); e) the relative portion of words consisting only of the symbols '0' and '2' (WPSUM02) and f) the relative portion of words consisting only of the symbols '1' and '3'. These indices are reported before and after injection (time = 0) with vehicle (V), and lipopolysaccharide (LPS). The black/white bar at the bottom indicates the dark/light periods, respectively. Data are shown as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ LPS vs. V, respectively, and + $p < 0.05$, + + $p < 0.01$ between basal levels (−3 h) of LPS and post-LPS challenge (pre-LPS vs. post-LPS) according to a Bonferroni posthoc test.

effects of LPS-induced endotoxemia using the same peripheral dose as employed here result in a considerable increment of the peripheral pro-inflammatory cytokines (Pacheco-Lopez et al., 2008). According to previous studies, we can then assume that some inflammatory cytokines such as the tumour necrosis factor-α (TNF-α), interleukin 1-β (IL-1β) and interleukin-6 (IL-6) remain elevated after +1 h and +7 h post-LPS injection (Mao and Huang, 2017; Pacheco-Lopez et al., 2008), which we here now associate with a lower complexity as indicated by the SD

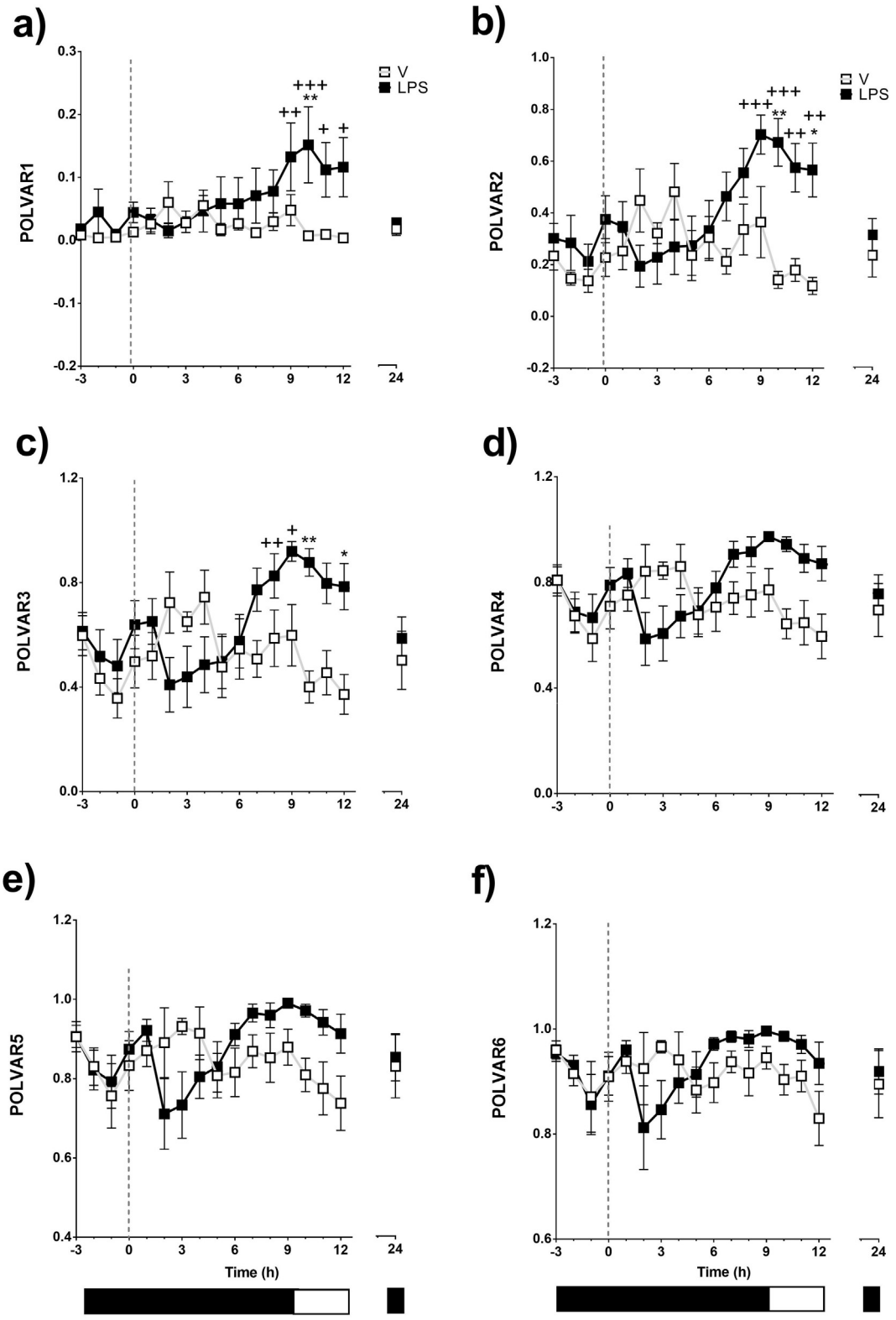

Fig. 3. Second symbolization or binary Δ-coding-method. Symbolic parameters of freely moving adult male Dark Agouti rats. The probability of low variability is represented by POLVAR values: a) 1 ms differences (POLVAR1); b) 2 ms differences (POLVAR2); c) 3 ms differences (POLVAR3); d) 4 ms differences (POLVAR4); e) 5 ms differences (POLVAR5) and f) 6 ms differences (POLVAR6). These indices are reported before and after injection (time = 0) with vehicle (V), and lipopolysaccharide (LPS). The black/white bar at the bottom of each panel indicates the dark/light periods, respectively. Data are shown as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ LPS vs. V, respectively, and + $p < 0.05$, + + $p < 0.01$, + + + $p < 0.001$ between basal levels (−3 h) of LPS and post-LPS challenge (pre-LPS vs. post-LPS) according to a Bonferroni posthoc test.

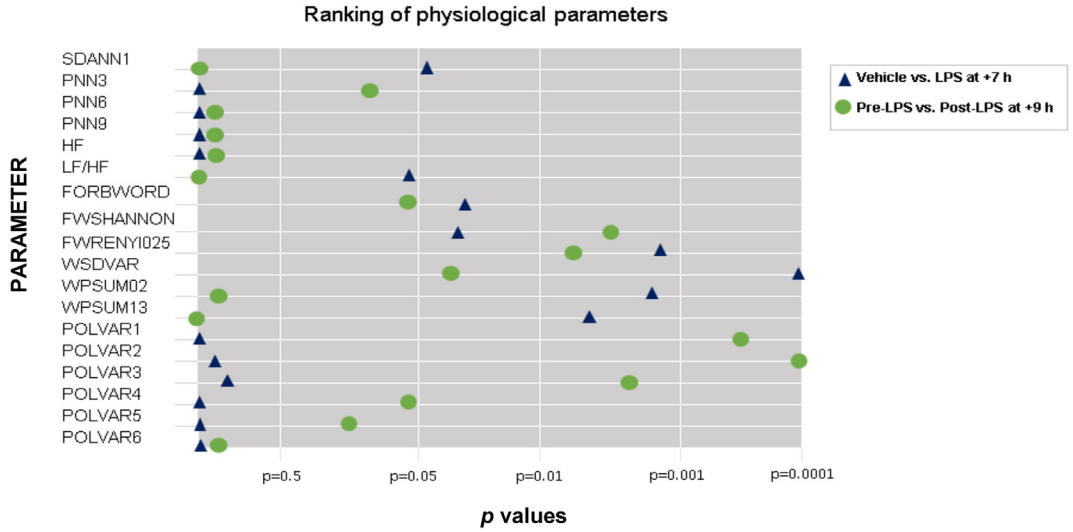

Fig. 4. The results of 18 different heart rate variability (HRV) indices to discriminate between V vs. LPS at +7 h and between pre- and post-LPS (+3 h vs. +9 h). The *p*-values shown are provided by the statistical analysis described in the corresponding section.

analysis of HRV (see Figs. 2 and 3). Another possibility considers HRV dynamics as a consequence of the interaction of different pro- and anti-inflammatory cytokines (Nikolic et al., 2013).

Noteworthy, the FWRENYI0.25 was the only parameter that presented an inverted biphasic behaviour characterized by a significant initial increment of complexity (at +2 h) followed by a long period of significantly lower complexity (at +7 h). This initial increase of complexity could be explained by the fast activation of the cholinergic anti-inflammatory pathway (CAP) in the presence of an acute inflammatory insult (Pavlov et al., 2003). Relevant findings indicate that the CAP activation is associated with an increased HRV (Pavlov et al., 2006). Moreover, concerning our FWRENYI0.25 long-period changes, other results suggest that a reduced HRV reflects a diminished cardiac vagal activity, thereby becoming a marker for a decreased activity of the CAP at the long term of the post-LPS injection (Huston and Tracey, 2011). Thus, the present study documents how the modifications of SD measures due to endotoxemia may function as autonomic biomarkers in further translational or clinical studies.

5. Limitations

We did not apply a nonlinearity test to evaluate the nonlinear dynamics of V and LPS groups. However, the presence of nonlinear dynamics in R-R fluctuations has been actually documented in rats (González et al., 2000), thereby supporting the use of SD analysis as applied here. Additionally, it is crucial to consider that either a cardiac control impairment or another type of dysfunction can affect the manifestation of nonlinear HRV dynamics (Silva et al., 2017b). Given the small sample size in the present study, our findings and interpretation should be confirmed in further explorations. Nonetheless, our previous studies have confirmed that even with a small number of cases ($N = 12$), it is possible to detect significant differences of peripheral cytokines levels in endotoxemic rats (Doenlen et al., 2011).

6. Conclusion

Our results show that SD seems to be more appropriate than a linear analysis to assess the course of the autonomic modulation of endotoxemic rats post-LPS injection. The corresponding SD indices detect decreased HRV and complexity, which indicate a modification of the autonomic regulation during LPS-induced endotoxemia. This modification is probably related to reduced activity of the CAP in the long term. These findings suggest that SD may help our understanding of the cardiovascular autonomic function during systemic inflammation.

Declaration of Competing Interest

The authors have no financial relationships relevant to this article to disclose.

The authors declare no conflict of interest.

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