Exogenous oxytocin reduces signs of sickness behavior and modifies heart rate fluctuations of endotoxemic rats

José Javier Reyes-Lagos [a], Martin Hadamitzky [b], Miguel Ángel Peña-Castillo [a], Juan Carlos Echeverría [a], Katharina Bösche [b], Laura Lückemann [b], Manfred Schedlowski [b], Gustavo Pacheco-López [c,d,*]

Abstract

Besides the well-known roles of oxytocin on birth, maternal bonding, and lactation, recent evidence shows that this hypothalamic hormone possesses cardioprotective, anti-inflammatory and parasympathetic neuromodulation properties. In this study, we explore the heart rate fluctuations (HRF) in an endotoxemic ro-dent model that was accompanied by the administration of exogenous oxytocin. The assessment of HRF has been widely used as an indirect measure of the cardiac autonomic function. In this context, adult male Dark Agou-ti rats were equipped with a telemetric transmitter to continuously and remotely measure the electrocardiogram, temperature, and locomotion. In a between-subjects experimental design, rats received the following peripheral treatment: saline solution as a vehicle (V); lipopolysaccharide (LPS); oxytocin (Ox); lipopolysaccharide + oxy-tocin (LPS + Ox). Linear and non-linear parameters of HRF were estimated starting 3 h before to 24 h after treat-ments. Our results showed that exogenous oxytocin does not modify by itself the HRF of oxytocin-treated rats in comparison to vehicle-treated rats. However, in animals undergoing endotoxemia it: a) provokes a less anticorrelated pattern in HRF, b) decreased mean heart rate, c) moderated the magnitude and duration of the LPS-induced hyperthermia, and d) increased locomotion, up to 6 h after the LPS injection. The less anticorrelated pattern in the HRF and decreased mean heart rate may reflect a cardiac pacemaker coupling with cholinergic in-fluences mediated by oxytocin during LPS-induced endotoxemia. Finally, the anti-lethargic and long-term tem-perature moderating effects of the administration of oxytocin during endotoxemia could be a consequence of the systemic anti-inflammatory properties of oxytocin.

Keywords:Heart rate variability; Sickness behavior; Áutonomic activity; Ánti-inflammatory cholinergic pathway; Oxytocin; LPS

1. Introduction

In healthy conditions, the cardiovascular fluctuations show complex physiological homeostatic dynamics resulting from interactions between the cardiac pacemaker cells and the autonomic nervous sys-tem [1]. Evidence in human and experimental animal models indicates that during systemic inflammation (e.g. endotoxemia) the regulation of the cardiac function is manifested with both decreased heart rate fluctu-ations (HRF) and increased cardiac rhythm regularity [2,3],possibly resulting from a partial uncoupling of the cardiac pacemaker cells from the autonomic neural control [4]. Some authors have also de-scribed that changes in the HRF can be used to indirectly identify the so called, cholinergic anti-inflammatory pathway (CAP) in diverse

---

[a] Metropolitan University (UAM), Campus Iztapalapa, Basic Sciences and Engineering Division, Mexico City 09340, Mexico
[b] University of Duisburg-Essen, University Hospital Essen, Institute of Medical Psychology and Behavioral Immunobiology, Essen 45122, Germany
[d] Metropolitan University (UAM), Campus Lerma, Biological and Health Sciences Division, Lerma 52005, Mexico
[d] Leiden University, Faculty of Social and Behavioral Sciences, Health, Medical and Neuropsychology Unit, Leiden, 2333, AK, The Netherlands

scenarios [5], and it has even been considered as a potential non-inva-sive tool to study and monitor the fetal inflammatory and anti-inflam-matory responses [6]. On this matter, a systemic inhibition of the α7 nicotinic acetylcholine receptor (α7nACHR) induced a decrement of the HRF by eliciting an increased inflammation and a febrile response in endotoxemic rats [7]. This response suggests a tonic role of peripheral nicotinic acetylcholine receptors for modulating heart rate dynamics during systemic inflammation.

Notwithstanding that oxytocin has well-known physiological func-tions during labor and lactation in humans and other mammals, studies have revealed that it has immune and cardioprotective properties as well; mainly by modulating the phasic activity of the parasympathetic nervous system [8]. In this regard, oxytocin possesses cardioprotective properties in ischemic conditions [9], and decreases carrageenan-in-duced inflammation in rats [10]. Additionally, the role of signal path-ways in cardioprotection have been shown to be stimulated by oxytocin receptors, which are present in both the heart and large vessels [11]. Evidence suggests that oxytocin also limits the cytokines activation caused by the bacterial cell-wall component lipopolysaccharide (LPS), possibly owing to the modulation of the CAP [12].

Further studies have reported other effects of the administration of intranasally oxytocin on HRF during social cognition experiments; where oxytocin, in addition to facilitate social behavior, decreases heart rate and increases HRF [13,14]. Other findings indicate that the subchronic administration of oxytocin induces long-lasting effects in ovariectomized rats, including an increase in spontaneous motor activ-ity and decreased levels of corticosterone [15].

We have recently reported linear and non-linear methods for the analysis of HRF during childbirth; a condition with was recently consid-ered to be accompanied by a conspicuous anti-inflammatory process [16]. In the same way, we have documented that the fractal scaling HRF analysis and sample entropy appeared as potential analytic tools that provide information regarding the homeostatic autonomic mecha-nisms related to inflammation [17]. In this context, the aim of the pres-ent study was to explore the effects of exogenous oxytocin in HRF dynamics by applying linear and non-linear analysis as well as motor activity and peripheral temperature in an experimental endotoxemia model in rats. Following the findings of Gholami et al. [4], Jankowski et al. [11] and Clodi et al. [12], we hypothesized that the peripheral ad-ministration of oxytocin during LPS-induced endotoxemia enhances a cardiac pacemaker coupling with cholinergic influences, which is reflected in the linear and non-linear parameters of the HRF, and re-duces signs of sickness behavior such as lethargy and fever.

2. Methods

2.1. Animals

Adult male Dark Agouti rats (DA/HanRj, 230–250 g) were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and housed in standard plastic cages with metal wire lids. Animals were maintained on a re-versed 12:12 h light/dark cycle (lights off at 7:00 AM) and had ad libitum access to water and standard diet. Rats were allowed to acclimate to the new surroundings for at least 2 weeks before transmitter implantation surgery. All procedures were followed in accordance with the animal fa-cilities of the University of Duisburg-Essen, Germany, and the experi-mental procedures were in accordance with the Animal Welfare Act (TierSchG) - Germany, the European Directive 2010/63/EU, and with the National Institutes of Health - USA Animal Care guidelines. These procedures were approved by the Institutional Animal Care and Use Committee (LANUV Düsseldorf, North Rhine-Westphalia, Germany).

2.2. Drugs

Based on a previous study [18], synthetic oxytocin (3 IU/ml, Rotexmedica GmbH, Arzneimittelwerk, Germany) was administered at a dose of 3 IU/kg (6 mcg/kg), subcutaneously (s.c.) in a volume of 1 ml. Lyophilized lipopolysaccharide (LPS) from Escherichia coli, sero-type O55:B5 (Sigma–Aldrich, Taufkirchen, Germany), was diluted with sterile saline (NaCl) and administered intraperitoneally (i.p.) at a dose of 0.1 mg/kg in a total volume of 1 ml. We and others have previously shown that this LPS dose induces a rise in peripheral cytokine levels and a moderate fever response [19], as well as changes of HRF parame-ters [7].

2.3. Telemeter implantation surgery

Telemetry transmitters (ETA-F20, Data Sciences International, St. Paul, Minnesota, USA) were surgically implanted for measuring contin-uously the electrocardiogram (ECG), peripheral temperature and loco-motor activity. Rats were anesthetized in an induction chamber with 5% isoflurane and 2% oxygen. Anesthesia was maintained by a flow of 1.5–2.0% isoflurane and oxygen (1 l/min) through a mask during sur-gery. The dorsal skin was cleaned and disinfected with povidone-iodine and alcohol. A 2 cm vertical incision was made in the dorsal skin, and the telemetry transmitter was placed s.c. The biopotential transmitter leads were tunneled under the skin with the tips guided to the right and left axillary regions. For pain relieve Carprofen 5 mg/kg was given s.c. on the day of surgery and the following three days. Experiments were con-ducted 14 days after surgery to allow recovery [20] .

2.4. Telemetric electrocardiogram recording in freely moving rats

Animals were randomly assigned to one of four different treatment groups: 1) vehicle (V, n = 7); 2) oxytocin (Ox, 3 IU/kg, n = 8); 3) lipo-polysaccharide (LPS, 0.1 mg/kg, n = 8); 4) LPS + oxytocin (LPS + Ox, combined administration of 0.1 mg/kg LPS and 3 IU/kg oxytocin, n = 8). After injections, rats were placed in their home cage with telemetry receivers placed below the cage and connected via a data exchange ma-trix to a PC. Baseline recordings started at 7:00 AM and the drugs admin-istration was performed at 10:00 AM (time 0) for each group. The ECG was sampled at 2000 Hz using Data Sciences International hardware and Dataquest ART software. This signal was visually inspected and re-liable 5-minute segments were selected each hour (−3 h to +24 h). All digitized signals were analyzed for ECG-QRS detection, temperature and activity assessment.

2.5. Data analysis

Raw ECG recordings were then processed using previously validated algorithms to generate the R-R interval or HRF series. These series were reconditioned by a filtering approach and processed as in previous stud-ies [21] to exclude for ectopic beats and artifacts.

Linear and non-linear parameters were calculated for each 5-min segment of the R-R interval data. The mean heart rate (mHR), as well as the root-mean-square of successive differences (RMSSD), which quantify vagally-mediated high frequency fluctuations, and the stan-dard deviation of the R-R intervals (SDNN), used as a measure of total HRF [22], were calculated for all series. Given that previous studies have shown that a linear frequency domain analysis does not provide additional information beyond that obtained from a time domain anal-ysis in endotoxemia experiments of rodents [23], we only performed a linear analysis in the time domain.

The short-term scaling exponents α_1, $\alpha_{1(MAG)}$, and $\alpha_{1(SIGN)}$ from $4 \leq n \leq 11$ beats and the long-term scaling parameters α_2, $\alpha_{2(MAG)}$, and $\alpha_{2(SIGN)}$ from n N 11 beats of all series were evaluated by applying detrended fluctuation analysis (DFA) and the magnitude and sign anal-yses (MSA) [24]. Values of the scaling exponents α_1 and α_2 of 0.5 indi-cate white noise and the absence of long-range correlations, a value of 1 reflects the behavior of a 1/f process having persistent long-range cor-relations whilst the slope of 1.5 indicates a random walk (Brownian noise) with a very smooth behavior where correlations still exist but do not follow in a power-law form [25].

For the MSA analysis, finding positive correlations in the magnitude series (i.e. α1(MAG) N 0.5) has been identified as reliable markers of non-linear properties [24].Theα1(SIGN) exponent provides information about the temporal directionality of the original series in relation to how se-ries' increments alternate, indicating if a positive or negative subse-quent increment (decrement) is more likely to occur given a current increment (decrement). To assess the regularity of the R-R time series we also estimated the sample entropy (SampEn) as described by Richman & Moorman [26]. The parameter m was fixed to 2, and toler-ance level r was 0.2.

The average actogram was calculated from continuous time series of body movement from −3 to +24 h using a software package for the analysis and visualization of chronobiological activity data [27].

2.6. Statistical analysis

All parameters were analyzed by two-way-ANOVA with repeated measures along time. According with the differences identified between groups, an area under curve (AUC) analysis was then applied at consec-utive 2 h periods (+7 to +9h). The AUC analysis was performed poste-rior to the time reported by Mazloom et al. [7] (i.e. after t = 6 h of the drugs or vehicle injections) and only during dark periods (i.e. before t = 9 h), with the intention of avoiding circadian variations induced by subsequent period of inactivity (light phase) [20]. However, with the goal of verifying further effects of oxytocin on LPS-induced endotoxemia, which seem to be manifested at long-term according to previous results reported by Clodi et al. [12], AUC data bars from body temperature were graphed and analyzed separately only from +7 to+9 h and +10 to +12 h corresponding to dark and light periods, re-spectively. The AUC for each period was calculated by the trapezoidal method (the AUC data are expressed as means ± SD). Finally, we made planned AUC comparisons between V vs. Ox, V vs. LPS and LPS vs. LPS + Ox groups by one-way ANOVA, followed by Fisher's LSD and Kolmogorov-Smirnov post hoc tests. (0.05 was considered as the signif-icance level).

3. Results

In Fig. 1 we show the estimated linear parameters of HRF starting 3 h before to 24 h after treatment. The AUC of each parameter associated to the +7 to +9 h period (shaded area) was compiled; their further anal-ysis is presented at the figure's insert. A black/white bar at the bottom of each panel indicates respectively the dark and light photoperiods. Our results show that LPS injection was associated with a biphasic dynamic characterized by an early increment in the mHR (Fig. 1a) and decremented SDNN (Fig. 1b) followed by a prolonged period of tachy-cardia and depressed HRF.

The effect of LPS on mHR was statistically significant according to planned comparisons of the AUC ($F = 19.28$; $p < 0.0001$, V vs. LPS). A single shot administration of oxytocin at a dose of 3 IU/kg provoked changes in the mHR 6 h post-LPS injection ($F = 19.28$; $p < 0.01$, LPS vs. LPS + Ox). The HRF linear indices SDNN (Fig. 1b) and RMSSD (Fig.1c) showed a significant reduction ($F = 7.52$; $p < 0.01$ and $F = 3.60$; $p < 0.05$, respectively) 6 h post-LPS injection.

The effect of the endotoxin injection on the irregularity structure of HRF series was also investigated using DFA and SampEn, depicted in Fig. 2. The short-term scaling exponent α1 remained decreased (Fig. 2a) from +7 to +9 h post-LPS injection according to the results of planned AUC comparisons ($F = 4.25$; $p < 0.01$, V vs. LPS).

The parameter α1(SIGN) (Fig. 2b) also presented a biphasic dynamic. From +7 to +9 h the LPS injection produced anticorrelated (lower) values in this parameter in comparison to vehicles ($F = 10.58$; $p < 0.0001$, V vs. LPS), but in contrast the LPS injection combined with oxytocin produced a less anticorrelated (higher) values in the endotoxemic rats ($F = 10.58$; $p < 0.01$, LPS vs. LPS + Ox).

The non-linear exponent α1(MAG) decreased significantly after the single dose of LPS ($F = 1.90$; $p < 0.05$, V vs. LPS), Fig. 2c. However, the SampEn parameter did not show changes from +7 to +9 h, Fig. 2d.

The long-term fractal scaling parameters (α2, α2(MAG),andα2(SIGN))did not present changes as well after drugs administration and they are not reported here.

In summary, a single dose administration of exogenous oxytocin did not modify by itself the heart rate dynamics from +7 to +9 h in any parameter (V vs. Ox); but in the presence of endotoxemia the oxytocin did introduce significant changes in the mHR and α1(SIGN)(LPS vs. LPS + Ox).

Fig. 3 shows representative examples of the linear and non-linear analysis of HRF of V, LPS, Ox, and LPS + Ox groups. The log–log F(n) vs. n and log–log F(n)/n vs. n relationships providing the α1, α1(SIGN), α1(MAG) exponents from a linear best-fit within the n range 4 to 11. There was linear relationship between log (fluctuation) and log (win-dow size) in all the groups at the time analyzed, which corresponds to fractal-like structure with 1/f dynamics [25].

Additionally, the Fig. 4 shows a sign decomposition of typical data for LPS and LPS + Ox groups. This figure illustrates a higher anticorrelated pattern in HRF during endotoxemia (LPS) in comparison to data from the LPS + Ox group.

Importantly, we also found that the circadian rhythm of peripheral body temperature is altered by LPS, as this group of rats presented an inverted dynamics in comparison to vehicle group (Fig. 5a). Furthermore a transient hypothermia from +2 to +4 h was observed in LPS-treated rats, the AUC corresponding to this period was smaller in relation to vehi-cle (70.9 °C h vs. 72.2 °C h respectively, with trending level of $F = 2.82$; $p = 0.06$).

Interestingly, oxytocin did not seem to have any effect on such transient hypothermia. Yet LPS administration induced a significant in-crement in body temperature from +10 to +12 h after treatment ($F = 9.65$; p b 0.001, V vs. LPS), which was significantly deregulated by the administration of oxytocin ($F = 9.65$; p b 0.05, LPS vs. LPS + Ox).

Finally, we found that exogenous oxytocin treatment reduced LPS-induced lethargy; as observed by significant changes in locomotion (Fig. 5b) at 6 h post-LPS (V vs. LPS, p b 0.003) and post-LPS and oxytocin injection (LPS vs. LPS + Ox, p b 0.05), according to the Kolmogorov-Smirnov non-parametric test.

4. Discussion

The results of this study demonstrate that a single exogenous dose of oxytocin does not modify by itself the HRF, temperature and body movement of oxytocin-treated rats in comparison to vehicle rats. How-ever, in animals undergoing endotoxemia this hormone provokes a less anticorrelated pattern in HRF indicated by $\alpha_{1(SIGN)}$ (Fig. 2b), decreased mHR (Fig. 1a), deregulation of peripheral temperature (Fig. 5a), and an increased locomotion (Fig. 5b) up to 6 h after the LPS injection.

A previous study showed that a single exogenous administration of oxytocin does not impact on the basal levels of corticotrophin, cortisol, tumor necrosis factor-α, and other cytokines [12]. In our study, the lack of HRF, temperature and body movement modifications after a sin-gle oxytocin administration is consistent with such study. However, physiological modulation introduced by oxytocin was only evident dur-ing endotoxemia (induced by LPS). In fact, Clodi et al. [12] suggested that exogenous oxytocin decreases the neuroendocrine and cytokine activation, and even activate the CAP, particularly during inflammation scenarios; supporting our data. Thus the significant changes in the mHR and α1(SIGN) identified 6 h after the administration of oxytocin, which re-veal less anticorrelated pattern in HRF and a decreased mean heart rate, suggest the occurrence of a cardiac pacemaker coupling with choliner-gic influences. This consideration is in line with the fact that some au-thors have considered that oxytocin embraces functional effects as a cardiovascular and autonomic modulating peptide [8,28,29];mainly mediated by local oxytocinergic receptors at the heart [30].Indeed, the administration of oxytocin could modulate α2-adrenoceptor bind-ing characteristics, thereby resulting in enhanced receptor activity [31].

Regarding the effects of the LPS injection, the results for the linear parameters of HRF (mHR and SDNN) as well as peripheral temperature between 0 to +6 h are consistent with a previous report [7].Inour rat model, we confirmed that the LPS injection induced a biphasic response pattern with an initial decrease in peripheral body temperature accom-panied by a prolonged period of increased mHR with reduced total HRF from 0 to +6 h. Whereas this study just reported data up to 6 h after treatment, our results also indicate that after that period the LPS effects were still evident on the HRF. We have previously characterized the im-munological outcome of an LPS peripheral administration at 0.1 mg/kg dose (identical dose as employed in this experiment); resulting in a sub-stantial increment of peripheral pro-inflammatory cytokines levels (tumor necrosis factor-α, interleukin 1-β

and interleukin 6) at 90 min after treatment [32] and central pro-inflammatory cytokines levels in the amygdala (tumor necrosis factor-α, interleukin 1-β) at 150 to 200 min after treatment [33]. Here, we also found that in the long-term (i.e. +7 to +9 h) the HRF remain affected by this single dose of LPS (as indicated by lower SDNN values, Fig. 1b). This effect on the HRF may then be associated with the continuation in the long-term of elevated pro-inflammatory cytokines levels as previously reported for shorter periods [33]. In fact, the SDNN has been associated with the peak expression of multiple cytokines by Fairchild et al. [23] as it shows an inverse correlation with tumor necrosis factor-α levels [34]. Continuing with the interpretation of linear HRF parameters, a potential vagal withdrawal or cholinergic uncoupling is observed for the LPS group (indicated by a reduced RMSSD, Fig. 1c). The vagal function is known to play a critical role in the regulation of the inflammatory response via the CAP because the vagal modulation is inversely associated with the inflammatory process [35]. Thus, when the vagal activity is low, the inhibitory influence on inflammation gets disrupted resulting in an excess of peripheral pro-inflammatory cytokines [36,37].

Concerning the non-linear HRF parameters, we observed a signifi-cant alteration in the HRF scaling dynamics and non-linear properties for the LPS group. This indicates that in response to endotoxin the frac-tal-like structure of cardiac periods is modified. Thus, the decrement of the α_1 and $\alpha_{1(MAG)}$ parameters (Fig. 2a and Fig. 2c, respectively) may in-dicate the uncoupling of the autonomic and cardiac systems [4].

The $\alpha_{1(SIGN)}$ short-term scaling parameter showed interesting changes, it was decremented after the LPS administration (Fig. 2b, V vs. LPS). The alternations of the R-R interval series can be visualized in the sign series; see example in Fig. 4a. These alternations indicate a stronger anticorrelated behavior (for both the R-R interval series and the sign series) under the influence of the LPS. Taking into consideration that the sign of the heartbeat increments is related to the interaction be-tween the sympathetic and the parasympathetic systems [38], such al-ternations should result from a sympathetic predominance during endotoxemia (reflected as well as an increased mHR, Fig. 1a). In fact, HRF spectral changes found in previous studies are consistent with a relative increase in sympathetic activity after LPS injection in adult animals [39]. Additional evidence supports that the scaling parameter $\alpha_{1(SIGN)}$ changes after the administration of an autonomic blocking drug in com-parison to a placebo group [38]. On the other hand, the exogenous oxy-tocin administration in conjunction with LPS produced a less anticorrelated pattern in the HRF during LPS-induced endotoxemia as reflected by an increased $\alpha_{1(SIGN)}$, see example in Fig. 4b, which is also manifested by a decreased mHR, Fig. 1a. The less anticorrelated pattern found in the LPS + Ox groups in comparison to LPS seems to be associ-ated with an amplitude increment in the HRF owing to the administra-tion of oxytocin.

By contrast, the linear RMSSD parameter failed to reveal differences between LPS vs. LPS + Ox groups. This restriction may be related to the manifestation of small RMSSD changes by our experimental design be-cause the parameter shows values near the resolution established by the ECG sample frequency (i.e. ≈ 0.5 ms). Yet, this restriction is not ap-preciated with the α1(SIGN) scaling exponent, becoming a potential index to identify the cardiac cholinergic control as similar reported elsewhere [17].

Given the well characterized “sickness behavior” induced by the LPS, we discard that the locomotor activity is the predominant factor explaining the changes of the HRF parameters reported here. Therefore, our results may be also explained by the activation of the inflammatory response after the LPS administration as identified by both a reduction of activity (Fig. 5b, V vs. LPS) and an increment of temperature (Fig. 5a, trending level of $F = 1.50$; $p = 0.07$). This interpretation is consis-tent with findings of studies showing that locomotion decreases follow-ing a LPS treatment in mice, thereby considering that the LPS administration may be used as a model for sickness-depression [40].

Our results for the peripheral temperature are in line with a previous study that reports the effects of oxytocin in combination with LPS in humans [12]. Similarly, the effects of oxytocin on temperature during endotoxemia seem to be manifested only toward the end of our obser-vation period (from +10 to +12 h). Therefore, these findings support the consideration that oxytocin might alleviate symptoms associated with systemic inflammation at long-term [41]. Noteworthy, Reid et al. compared the core temperature and the peripheral temperature after LPS

injection in Holstein steers, which lead them to suggest that the core temperature and the peripheral temperature do not move in syn-chrony after an LPS challenge, rather both follow an inverse relation [42]. In this sense, our results for temperature may have been influenced by the transmitters' location (i.e. subcutaneously implanted), conse-quently more studies are still necessary to elucidate the influences of oxytocin in response to LPS-challenge on the regulation of body temperature.

Finally, locomotion was also influenced by the single dose of oxyto-cin (Fig. 5b, LPS vs. LPS + Ox) that restricted the lethargic LPS-induced effects. This condition may indirectly reflect the anti-inflammatory properties of oxytocin. Indeed, previous evidence suggests that a sub-chronic administration of oxytocin reduces fatigue in older adults [43] and increases the effects of clonidine (an alpha 2-adrenoreceptor ago-nist) by reducing blood pressure and modifying the locomotor activity in rats [44]. Although oxytocin has a half-life of only few minutes, the re-duction in blood pressure in response to oxytocin has not been observed until about 6 to 8 h after its first administration [44]. These cardiovascu-lar effects are coherent and timely coincident with the delayed changes introduced by oxytocin on rats' locomotion and HRF parameters during the LPS-induced endotoxemia of our study.

4.1. Limitations

It should acknowledge that we did not test different doses of oxyto-cin that may modify our results. However, with the administration of a single low dose of oxytocin we were able to introduce changes in fractal and non-linear parameters of HRF during endotoxemia. Yet, owing to transmitter's model used here and its actual location, the core tempera-ture and blood pressure were not possible to be recorded. In fact, previ-ous research suggests that oxytocin and LPS both decrease blood pressure in rodents [45,46], and lower values of HRF have also been as-sociated to higher values of blood pressure [47]. Another indicates that LPS produces a rapid impairment of the baroreflex function [48], inde-pendently from the level of blood pressure, while oxytocin is likely act-ing on the baroreceptor reflex sensitivity as well [49]. Future work will consider applying higher doses of oxytocin as well as monitoring the cholinergic spillovers, blood pressure and core temperature and trying other routes of administration to investigate the actual relationship be-tween peripheral cytokines levels and HRF parameters.

5. Conclusion

During LPS-induced endotoxemia the long-term HRF became more anticorrelated (as indicated by lower values of $\alpha_{1(SIGN)}$). In addition, this endotoxemia was accompanied by: a) tachycardia, b) a loss of frac-tal heart rate dynamics, c) changes in the peripheral temperature, and d) lethargic behavior. However, a concomitant single dose of peripheral oxytocin provoked a less anticorrelated pattern in the long-term HRF (as indicated by higher values of $\alpha_{1(SIGN)}$), restored the normal heart rate values, reduced lethargy and moderated the LPS-induced hyper-thermia. We suggest that the less anticorrelated pattern in the HRF and decreased mean heart rate may result from a cardiac cholinergic au-tonomic coupling owing to both the concomitant administration of sys-temic exogenous oxytocin during LPS-induced endotoxemia and its interaction with heart's oxytocin receptors.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgments

J. Javier Reyes-Lagos thanks the Mexican Council for Science and Technology (CONACyT) for providing scholarship (CVU/Scholarship number: 381983/253449). This work was partially funded by the Met-ropolitan University (UAM)- Mexico research and mobility funds to GPL, MAPC and JCE, as well as institutional funds of the University Hos-pital of Essen- Germany.

References

[1] L. Glass, Introduction to controversial topics in nonlinear science: is the normal heart rate chaotic? Chaos 19 (2009), http:// dx doi.org/10.1063/1.3156832.

[2] P.J. Godin, L.A. Fleisher, A. Eidsath, R.W. Vandivier, H.L. Preas, S.M. Banks, et al., Ex-perimental Human Endotoxemia Increases Cardiac Regularity: Results From a Pro-spective, Randomized, Crossover Trial, 1996, http:// dx doi.org/10.1097/00003246-199607000-00009.

[3] K.D. Fairchild, V. Srinivasan, J.R. Moorman, R.P.A. Gaykema, L.E. Goehler, Pathogen-induced heart rate changes associated with cholinergic nervous system activation, Am. J. Phys. Regul. Integr. Comp. Phys. 300 (2011) R330–R339, http://dx.doi.org/ 10.1152/ ajpregu.00487.2010.

[4] M. Gholami, P. Mazaheri, A. Mohamadi, T. Dehpour, F. Safari, S. Hajizadeh, et al., Endotoxemia is associated with partial uncoupling of cardiac pacemaker from cho-linergic neural control in rats, Shock 37 (2012) 219–227, http://dx doi.org/10.1097/ SHK.0b013e318240b4be.

[5] J.M. Huston, K.J. Tracey, The pulse of inflammation: heart rate variability, the cholin-ergic anti-inflammatory pathway and implications for therapy, J. Intern. Med. 269 (2011) 45–53, http://dx.doi.org/10.1111/j.1365-2796.2010.02321 x.

[6] L.D. Durosier, C.L. Herry, M. Cortes, M. Cao, P. Burns, A. Desrochers, et al., Does heart rate variability reflect the systemic inflammatory response in a fetal sheep model of lipopolysaccharide-induced sepsis? Physiol. Meas. 36 (2015) 2089–2102, http://dx. doi.org/10.1088/0967-3334/36/10/2089.

[7] R. Mazloom, G. Eftekhari, M. Rahimi, V. Khori, S. Hajizadeh, A.R. Dehpour, et al., The role of α7 nicotinic acetylcholine receptor in modulation of heart rate dynamics in endotoxemic rats, PLoS One 8 (2013) 1–14, http://dx.doi.org/10.1371/journal. pone.0082251.

[8] M. Gamer, C. Büchel, Oxytocin specifically enhances valence-dependent parasympa-thetic responses, Psychoneuroendocrinology 37 (2012) 87–93, http://dx doi.org/10. 1016/j psyneuen.2011.05.007.

[9] M. Jankowski, V. Bissonauth, L. Gao, M. Gangal, D. Wang, B. Danalache, et al., Anti-in-flammatory effect of oxytocin in rat myocardial infarction, Basic Res. Cardiol. 105 (2010) 205–218, http://dx.doi.org/10 1007/s00395-009-0076-5.

[10] M. Petersson, U. Wiberg, T. Lundeberg, K. Uvnäs-Moberg, Oxytocin decreases carra-geenan induced inflammation in rats, Peptides 22 (2001) 1479–1484, http://dx.doi. org/10.1016/S0196-9781(01)00469-7.

[11] M. Jankowski, D. Wang, F. Hajjar, S. Mukaddam-Daher, S.M. McCann, J. Gutkowska, Oxytocin and its receptors are synthesized in the rat vasculature, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 6207–6211, http://dx.doi.org/10.1073/pnas.110137497

[12] M. Clodi, G. Vila, R. Geyeregger, M. Riedl, T.M. Stulnig, J. Struck, et al., Oxytocin alle-viates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E686–E691, http://dx.doi.org/ 10.1152/ ajpendo 90263.2008.

[13] A. Kis, O. Kanizsár, M. Gácsi, J. Topál, Intranasally administered oxytocin decreases heart rate and increases heart rate variability in dogs, J. Vet. Behav. Clin. Appl. Res. 9 (2014), e15, http://dx.doi.org/10.1016/j.jveb.2014.09.050.

[14] A.H. Kemp, D.S. Quintana, R.L. Kuhnert, K. Griffiths, I.B. Hickie, A.J. Guastella, Oxyto-cin increases heart rate variability in humans at rest: implications for social ap-proach-related motivation and capacity for social engagement, PLoS One 7 (2012) 3–8, http:// dx doi.org/10.1371/journal.pone 0044014.

[15] M. Petersson, M. Eklund, K. Uvnäs-Moberg, Oxytocin decreases corticosterone and nociception and increases motor activity in OVX rats, Maturitas 51 (2005) 426–433, http://dx.doi.org/10.1016/j.maturitas.2004.10 005.

[16] J.J. Reyes-Lagos, J.C. Echeverría-Arjonilla, M.A. Peña-Castillo, A.J. Montiel-Castro, G. Pacheco-López, Physiological, immunological and evolutionary perspectives of labor as an inflammatory process, Adv. Neuroimmune Biol. 5 (2014) 75–89, http://dx.doi.org/10.3233/ NIB-140085.

[17] J.J. Reyes-Lagos, J.C. Echeverría-Arjonilla, M.Á. Peña-Castillo, M.T. García-González, M.D.R. Ortiz-Pedroza, G. Pacheco-López, et al., A comparison of heart rate variability in women at the third trimester of pregnancy and during low-risk labour, Physiol. Behav. 149 (2015) 255–261, http://dx doi.org/10.1016/j.physbeh.2015.05.041

[18] J.W. Carpenter, Exotic Animal Formulary, third ed. Saunders, St. Louis (MO), 2004.

[19] H. Engler, R. Doenlen, C. Riether, A. Engler, M.B. Niemi, H.O. Besedovsky, et al., Time-dependent alterations of peripheral immune parameters after nigrostriatal dopa-mine depletion in a rat model of Parkinson's disease, Brain Behav. Immun. 23 (2009) 518–526, http://dx doi.org/10.1016/j.bbi.2009.01.018.

[20] J. Thireau, B.L. Zhang, D. Poisson, D. Babuty, Heart rate variability in mice a theoret-ical and practical guide, Exp. Physiol. 93 (2008) 83–94, http://dx doi.org/10.1113/expphysiol.2007.040733.

[21] N. Wessel, A. Voss, H. Malberg, C. Ziehmann, H.U. Voss, A. Schirdewan, et al., Nonlinear analysis of complex phenomena in cardiological data, Herzschrittmachertherapie Und Elektrophysiologie. 11 (2000) 159–173, http://dx doi.org/10.1007/s003990070035

[22] M. Mal k, J. Bigger, A. Camm, R. Kleiger, Heart rate variability. Standards of measure-ment, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiol-ogy, Eur. Heart J. 17 (1996) 354–381 (doi:0195-668X/96/030354+28$18.00/0).

[23] K.D. Fairchild, J.J. Saucerman, L.L. Raynor, J.A. Sivak, Y Xiao, D.E. Lake, et al., Endotox-in depresses heart rate variability in mice cytokine and steroid effects, Am. J. Phys Regul. Integr. Comp. Phys. 297 (2009) R1019–R1027, http://dx.doi.org/10 1152/ ajpregu.00132.2009.

[24] Y. Ashkenazy, S. Havlin, P.C. Ivanov, C.K. Peng, V. Schulte-Frohlinde, H.E. Stanley, Magnitude and sign scaling in power-law correlated time series, Phys. A Stat. Mech. Its Appl. 323 (2003) 19–41, http://dx.doi.org/10.1016/S0378-4371(03)00008-6

[25] C.K. Peng, S. Havlin, H.E. Stanley, A.L. Goldberger, Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series, Chaos 5 (1995) 82–87, http://dx.doi.org/10 1063/1.166141.

[26] J.S. Richman, J.R. Moorman, Physiological time-series analysis using approximate entropy and sample entropy, Am. J. Physiol. Heart Circ. Physiol. 278 (2000) H2039–H2049.

[27] B. Schmid, C. Helfrich-Förster, T. Yoshii, A new ImageJ plug-in "ActogramJ" for chro-nobiological analyses, J. Biol. Rhythm. 26 (2011) 464–467, http://dx doi.org/10. 1177/0748730411414264.

[28] K.T. Higa, E. Mori, F.F. Viana, M. Morris, L.C. Michelini, Baroreflex control of heart rate by oxytocin in the solitary-vagal complex, Am. J. Phys Regul. Integr. Comp Phys. 282 (2002) R537–R545, http //dx doi.org/10.1152/ajpregu.00806.2000.
[29] G.J. Norman, J.T. Cacioppo, J.S. Morris, W.B. Malarkey, G.G. Berntson, A.C. DeVries, Oxytocin increases autonomic cardiac control: moderation by loneliness, Biol. Psychol. 86 (2011) 174–180, http://dx.doi.org/10.1016/j.biopsycho.2010 11 006.
[30] N.J. Cicutti, C.E. Smyth, O.P Rosaeg, M. Wi kinson, Oxytocin receptor binding in rat and human heart, Can J Cardiol. 15 (1999) 1267 1273.
[31] Z. Diaz-Cabiale, M. Petersson, J.A. Narvaez, K. Uvnas-Moberg, K. Fuxe, Systemic oxy-tocin treatment modulates α2-adrenoceptors in telencephalic and diencephalic re-gions of the rat, Brain Res. 887 (2000) 421–425, http://dx doi.org/10.1016/S0006-8993(00)03017-1.
[32] G. Pacheco-López, M.B. Niemi, H. Engler, A. Engler, C. Riether, R. Doenlen, et al., Weaken taste-LPS association during endotoxin tolerance, Physiol. Behav. 93 (2008) 261–266, http·//dx.doi.org/10.1016/j physbeh.2007 08 022.
[33] H. Engler, R. Doenlen, A. Engler, C. Riether, G. Prager, M.B. Niemi, et al., Acute amyg-daloid response to systemic inflammation, Brain Behav. Immun. 25 (2011) 1384–1392, http://dx doi.org/10.1016/j.bbi.2011.04.005.
[34] V.N. Nikolic, T. Jevtovic-Stoimenov, D. Stokanovic, M. Milovanovic, R. Velickovic-Radovanovic, S. Pesic, et al., An inverse correlation between TNF alpha serum levels and heart rate variability in patients with heart failure, J. Cardiol. 62 (2013) 37–43, http // dx doi.org/10.1016/j.jjcc.2013.02.013.
[35] V.A. Pavlov, H. Wang, C.J. Czura, S.G. Friedman, K.J. Tracey, The cholinergic anti-in-flammatory pathway a missing link in neuroimmunomodulation, Mol. Med. 9 (2003) 125–134.
[36] V.A. Pavlov, Cholinergic modulation of inflammation, Int. J. Clin. Exp Med. 1 (2008) 203–212.
[37] I. Zila, D. Mokra, J. Kopincova, M. Kolomaznik, M. Javorka, A. Calkovska, Heart rate variability and inflammatory response in rats with lipopolysaccharide-induced endotoxemia, Physiol. Res. 64 (Suppl. 5) (2016) S669–S676 (http://www.ncbi. nlm nih gov/ pubmed/26674290).
[38] Y. Ashkenazy, P.C. Ivanov, S. Havlin, C.K. Peng, Y. Yamamoto, A.L. Goldberger, et al., Decomposition of heartbeat time series: scaling analysis of the sign sequence, Comput. Cardiol. 27 (2000) 139–142, http://dx.doi.org/10.1109/CIC.2000.898475.
[39] J. Huang, Y. Wang, D. Jiang, J. Zhou, X. Huang, The sympathetic-vagal balance against endotoxemia, J. Neural Transm. 117 (2010) 729–735, http //dx.doi.org/10.1007/s00702-010-0407-6.
[40] C.G. Engeland, D.V. Nielsen, M. Kavaliers, K.P. Ossenkopp, Locomotor activity chang-es following lipopolysaccharide treatment in mice: a multivariate assessment of be-havioral tolerance, Physiol. Behav. 72 (2001) 481–491, http //dx doi.org/10.1016/ S0031-9384(00)00436-4.
[41] L. Yuan, S. Liu, X. Bai, Y. Gao, G. Liu, X. Wang, et al., Oxytocin inhibits lipopolysaccha-ride-induced inflammation in microglial cells and attenuates microglial activation in lipopolysaccharide-treated mice, J. Neuroinflammation 13 (2016) 77, http://dx.doi. org/10.1186/ s12974-016-0541-7.
[42] E.D. Reid, K. Fried, J.M. Velasco, G.E. Dahl, Correlation of rectal temperature and pe-ripheral temperature from implantable radio-frequency microchips in Holstein steers challenged with lipopolysaccharide under thermoneutral and high ambient temperatures, J. Anim. Sci. 90 (2012) 4788–4794, http //dx doi.org/10.2527/jas. 2011-4705.
[43] J.A. Barraza, N.S. Grewal, S. Ropacki, P Perez, A. Gonzalez, P.J. Zak, Effects of a 10-day oxytocin trial in older adults on health and well-being, Exp. Clin. Psychopharmacol. 21 (2013) 85–92, http://dx.doi.org/10.1037/a0031581.
[44] M. Petersson, T. Lundeberg, K. Uvnäs-Moberg, Oxytocin enhances the effects of clo-nidine on blood pressure and locomotor activity in rats, J. Auton. Nerv. Syst. 78 (1999) 49–56, http //dx.doi.org/10.1016/S0165-1838(99)00061-2.
[45] M. Petersson, P. Alster, T. Lundeberg, K. Uvnäs-Moberg, Oxytocin causes a long-term decrease of blood pressure in female and male rats, Physiol. Behav. 60 (1997) 1311–1315, http://dx doi.org/10.1016/S0031-9384(96)00261-2.
[46] S. Ehrentraut, S. Frede, H. Stapel, T. Mengden, C. Grohé, J. Fandrey, et al., Antagonism of lipopolysaccharide-induced blood pressure attenuation and vascular contractility, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2170–2176, http://dx doi.org/10.1161/ ATVBAHA.107.146100.
[47] M.F. Lutfi, M Y. Sukkar, Effect of blood pressure on heart rate variability, Khartoum Med. J. 04 (2011) 548–553.
[48] A. Radaelli, P. Castiglioni, C. De Carlini, F. Soriano, M. Di Rienzo, G. Mancia, Infusion of E. coli lipopolysaccharides toxin in rats produces a severe impairment of baroreflex function in absence of blood pressure changes, J. Hypertens. 28 (2010), e528.
[49] C. Vela, Z. Diaz-Cabiale, C. Parrado, M. Narvaez, R. Covenas, J.A. Narvaez, Involvement of oxytocin in the nucleus tractus solitarii on central cardiovascular control: interac-tions with glutamate, J. Physiol. Pharmacol. 61 (2010) 59–65.