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Shifts in the light-dark cycle increase unpredictability of the cardiovascular system

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ABSTRACT

Physiological variables such as heart rate (HR) and blood pressure (BP) exhibit long-term circadian rhythms, which can be disturbed by shift work. On the other hand, short-term oscillations in HR and BP have a high prognostic value. Therefore, we aimed to determine if the short-term variability, complexity and entropy of HR and BP would be affected by a regular light/dark (LD) cycle and phase delay shifts of the LD cycle, leading to chronodisruption. Telemetry-monitored rats were exposed first to the regular LD cycle and then to shifts in LD for 8 weeks. On the basis of long-term HR and BP recording and evaluation, we found circadian rhythms in HR and BP variability, complexity and entropy under regular LD cycles. Short-term exposure to shifts disturbed circadian rhythms of HR and BP variability, complexity and entropy, indicating chronodisruption. The power of circadian rhythms was suppressed after 8 weeks of phase delay shifts. Long-term exposure to shifts increased variability ($p = 0.007$), complexity ($p < 0.001$) and dark-time entropy ($p = 0.006$) of HR but not BP. This is the first study demonstrating long-term recording and estimation of HR and BP variability, complexity and entropy in conscious rats exposed to irregular lighting conditions. After long-term phase delay shifts, short-term variability of HR was less predictable than in controls. This study suggests that changes in short-term HR and BP oscillations induced by long-term shift work can negatively affect cardiovascular health.

1. Introduction

Physiological variables oscillate over time, during both the active and passive phases of the day (Blum et al., 2014). Therefore, not only absolute values of physiological outcomes but also their changes and oscillations in different time ranges are very important (Hermida et al., 2007). For instance, the short-term variability in heart rate (HR) and blood pressure (BP) are changed under conditions of anxiety and depression (Chalmers et al., 2014) and can serve as a predictor of a worsened cardiovascular prognosis (Goya-Esteban et al., 2010; Chen et al., 2016).

Physiological oscillations can be evaluated by time-domain, frequency-domain and nonlinear methods (Aubert et al., 2009; Tanev et al., 2014; Young and Benton, 2015). The time-domain methods are the simplest to perform. They evaluate physiological data with respect to time and measure standard deviation within successive HR or RR interval series or its root mean square (Kleiger et al., 2005).

The frequency-domain methods reflect the magnitude of HR and BP variability. They are divided into low- (LF) and high-frequency (HF) bands that are simplistically modulated by different branches of the autonomic nervous system (Ning et al., 2006). For HR variability, the LF range (0.20–0.75 Hz in rats) primarily reflects the sympathetic efferent modulation of the sinoatrial node and/or vagal system (Japundzic et al., 1990; Ning et al., 2006; Santos et al., 2015), while HF (0.75–2.50 Hz in rats) is affected by breathing and vagal activity (Ning et al., 2006). On the other hand, the sympathetic and myogenic activity of vascular tone modulates the LF band of BP variability, while the HF band of BP variability is impaired by mechanical factors such as stroke volume, cardiac output and respiration (Almog et al., 1998; Ning et al., 2006; Santos et al., 2015). Since variability in HR and BP are non-stationary (Hsieh et al., 2014; Tanev et al., 2014), evaluation of the variability requires a combination of spectral analysis with other techniques other than linear analyses. For these purposes nonlinear analyses are considered as appropriate tools (Hsieh et al., 2014; Silva et al., 2017; Tanev et al., 2014).

Sample entropy (SampEn) belongs to nonlinear analyses. It provides an improved evaluation of time-series regularity and is a useful tool in studies of the dynamics of human cardiovascular physiology (Rajendra Acharya et al., 2006). Reduced entropy indicates greater regularity of physiological systems and is connected with a higher incidence of diseases and aging (Dimitriev et al., 2016; Pincus and Viscarello, 1992). Moreover, SampEn values are able to predict the percentage of atrial

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fibrillation in humans (Sadrawi et al., 2016).

Another nonlinear parameter, detrended fluctuation analysis (DFA), evaluates the complexity and the presence or absence of fractal correlation properties in beat-to-beat data (de Souza et al., 2014). In cardiovascular outcomes such as HR and BP, time scale-invariant (fractal) patterns are present (Goldberger et al., 2002), which can be affected by cardiovascular diseases (de Souza et al., 2014; Goldberger et al., 2002; Ivanov et al., 1999). Moreover, these patterns can predict clinical outcomes such as survival rate assessment (Peng et al., 1995a, 1995b).

In addition to short-term variability in heart rate (HR) and blood pressure (BP), cardiovascular parameters exhibit prominent circadian rhythms (Scheer et al., 2003). Circadian rhythms are generated by the suprachiasmatic nucleus (SCN), which is also involved in the fractal regulation of behavioural activity fluctuation in humans and rats (Hu et al., 2008) and can generate oscillations from approximately 1 min to 10 h (Hu et al., 2012). Circadian rhythms can be disturbed by shift work or jet lag; thus, the SCN could be potentially involved in the progression of cardiovascular diseases (Puttonen et al., 2010). In shift workers, circadian variation in cardiac autonomic variation is not in phase with sleep-wake cycles (Yoshizaki et al., 2013a). Moreover, shift-work or SCN lesions disrupt circadian and fractal patterns of activity (Hsieh et al., 2014). However, experimental data showed that several weeks of shifts did not alter ultradian (several hours) oscillations of HR and BP (Molcan et al., 2013). As far as we know cardiovascular data about short-term (beat-to-beat) responses during long-term circadian disruption are missing.

Therefore, we aimed to reveal if short-term variability, complexity and entropy of HR and BP oscillations exhibit circadian rhythms and if these circadian rhythms respond to long-term phase delay shifts in the LD cycle.

2. Materials and methods

2.1. Animals and ethics statement

Normotensive, mature male Wistar rats (n = 4; 16 weeks old; 300 ± 19 g at the beginning of the experiment) were used in this study. Animals were kept alone in plastic cages with food and water *ad libitum* under a stable 12 h light-12 h dark cycle (LD 12:12; lights on at 06:00; intensity 150 lx), with controlled room temperature (21 ± 2 °C) and humidity (55 ± 10%). The study was approved by the Ethics Committee for the Care and Use of Laboratory Animals at the Comenius University in Bratislava, Slovak Republic and the State Veterinary Authority of Slovak Republic.

2.2. Heart rate and blood pressure monitoring

The surgery was performed under isoflurane anaesthesia (induction: 4% isoflurane in 100% oxygen; maintained: 1.5%–2% isoflurane in 100% oxygen). The rat telemetry transmitters (TA11PA-C40; Data Sciences International, St. Paul, MN, USA) were surgically implanted into the abdominal aorta just above its bifurcation (Brockway et al., 1991; Svitok et al., 2016). The catheter was stabilised in the aorta with tissue glue (3 M Vetbond; DSI, MN, USA) and covered with a cellulose patch (Cellulose Patch Kit Small Animals; DSI, MN, USA). Rats were treated postoperatively with ampicillin (100 mg/kg; s.c.; BB Pharma a.s., Prague, Czech Republic) and tramadol (15 mg/kg; s.c.; Tramal, Stada, Bad Vilbel, Germany). Two weeks after the surgery, when distinct circadian rhythms of BP and HR were present, animals were initiated into the study.

2.3. Experimental design

Telemetry-monitored animals were exposed to the phase-delay shifts of LD cycle (PDS) with the dark phase extended in 8-h every 2 days over 8 weeks (Fig. 1). This schedule is described in details in our previous study focused on endocrine and cardiovascular rhythms in phase delayed rats (Zeman et al., 2016). Five-minute segments of pressure signal were continuously acquired (Dataquest A.R.T. 4.31 gold version; DSI, MN, USA) 5 min with a sampling frequency of 500 Hz every 15 min during the study. From the original pressure signal, HR (beat-to-beat [ms]) and systolic BP were detected and exported as .txt files. HR and systolic BP data were subjected to analysis at the control LD, first (S01) and last (S08) experimental PDS weeks (Fig. 1). Frequency-domain (LF/HF) and nonlinear (DFA α1 and α2 scaling exponent and SampEn) analyses were performed in HRV Analysis Software (Ramshur, 2010).

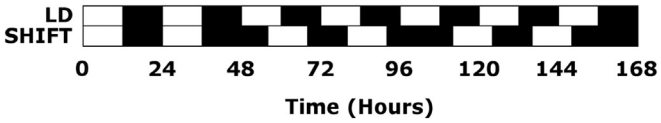

Fig. 1. Design of the study. Rats were kept at 12L:12D during one control week followed by 8 weeks of phase delay shifts in the LD cycle (PDS).

2.4. Frequency-domain analysis

Data segments with a > 5% ectopic beats were removed from the data cluster. Trends in measured data were removed by continuous wavelet transformation. To minimise spectral leakage, segments were interpolated (5 Hz) with 50% overlapping windows. Frequency-domains were calculated by the Lomb-Scargle periodogram, divided into LF (0.2–0.75 Hz) and HF (0.75–2.5 Hz) bands and normalised. The ratio (LF/HF) was used as an index of sympathovagal balance (Ning et al., 2006).

2.5. Nonlinear analysis

DFA is a promising method to detect dynamic changes in cardiovascular variables under different physiological and clinical conditions (Gomes et al., 2002; Mäkikallio et al., 1998), because it is not affected by the lack of stationarity (Peng et al., 1995a, 1995b). Fluctuations in correlations embedded in time series of HR and BP can be characterised by the scaling exponent α (the slope of the regression line). In our study, we evaluated short-term (α1; 4–13 beats) and long-term (α2; 14–100 beats) fluctuation slope. Briefly, α = 0.5 indicates white noise; α = 1.5 resembles brown noise and if 0.5 < α < 1.5, there are positive correlations. If α is close to 1.0, it indicates a more complex (nonlinear) system, and if it reaches values above 1.0, the system tends to be less complex and linear (de Souza et al., 2014), indicating a bad prognosis or inadequate physiology (Mäkikallio et al., 1999).

SampEn measures the complexity of irregular signals. It is similar to approximate entropy with some differences in calculation (Richman and Moorman, 2000). SampEn estimates the negative natural logarithm of the conditional probability that two sequences similar for m points remain similar for (m + 1) points, within a tolerance (r), excluding self-matches (Dimitriev et al., 2016; Lake et al., 2002). We set the pattern length parameter (m) as 3 and the tolerance level (r) for determining of differences between data points as 0.1 SD (Dimitriev et al., 2016; Gonçalves et al., 2010). The highest value of SampEn means that the data are more irregular, complex and more unpredictable.

2.6. Circadian rhythm analysis

Circadian rhythms in physiological variables are not strictly aligned to the light/dark regimen. Values can change before L/D transition and thus L/D differences can be suppressed in comparison with amplitude of circadian rhythms. Therefore, in conscious, freely moving animals, we estimated the presence of circadian rhythms, the power of circadian rhythms (F of significant 24-h period), acrophase (the time at which the peak of a rhythm occurs) and the amplitude [the difference between