Spectral Analysis of Cardiovascular Parameters of Rats Under Irregular Light-Dark Regime

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Abstract.
Our study is related to shift work that possibly brings negative consequences on public health. Adaptation of cardiovascular parameters in rats to rotating phase delay shift in light regime was addressed. Prolongation of circadian rhythms was observed for blood pressures, heart rate and locomotive activity. Relative amount of circadian in comparison to ultradian rhythms was strongly activated by shifted regime in its first weeks, while being the lowest during persistent light conditions. Results contribute to understanding of physiological changes that accompany shift work.

Keywords: spectral analysis, Lomb-Scargle periodogram, unevenly sampled data

1. Introduction

Work connected with shifted day regime is a common feature of modern society. However, it can alter biological rhythms in humans. Chronic disturbances of biological clocks results in the suppression of circadian rhythms with possible negative consequences on the cardiovascular, neuroendocrine and gastrointestinal system. Such regime can contribute to the development of many neurological, cardiovascular [1] and cancer diseases [2].

In epidemiologic studies many factors are usually simultaneously involved, thus they can hardly elucidate all negative effects of shift work on physiologic parameters or reveal hidden causal interrelationships. Moreover, results of epidemiological studies linking shift work and various health risk factors often claim opposite effects. Therefore it is the advantage of animal model with treatment under controlled conditions, which can analyze different factors separately. Experimental studies using animal models are needed for better understanding of biological mechanisms of endogenous biological clocks and the way how they control variety of body functions.

Biological clocks control physiological and behavioural processes in all organisms, including humans. Regular synchronization is a necessary prerequisite for their normal function. Light is the most significant stimulus for resetting biological clocks in higher vertebrates [3]. The light signal is transferred from the retina to the central oscillator via the retinohypothalamic tract. In the central oscillator a rhythmic, circadian, transcription of clock genes occurs and the signal is transferred by output pathways into all organs of the body. In this work we assessed effects of light regime as the main factor, which can affect the central oscillator, on the control of cardiovascular system of rats.

The aim of this study was to analyze the effect of irregular light-dark regime on endogenous circadian and ultradian rhythms of selected physiological parameters in rats. In this part of the work cardiovascular parameters and locomotive activity are addressed. In particular, we investigated how rotating phase delay shifts impact rhythmic changes of systolic and diastolic blood pressure (SP and DP), hearth rate (HR) and locomotive activity (LA) of rats.

2. Subject and Methods

Cardiovascular and locomotive variables were measured telemetrically using the telemetric sensor TA 11 PA-C40 (DSI St. Paul, Minnesota, USA). Monitoring begun two weeks after the surgical implantation of sensors into the abdominal aorta of 4 female normotensive rat *(Rattus norvegicus,*12 month old of the beginning of the experiment). During a time span of 18 weeks the rats were exposed to the following set of conditions: control 12:12 light/dark (LD) for a period of one week; phase delay shifts of light-dark regime by eight hours every second day (Shift) for a period of 12 weeks; again LD 12:12 regime for one week; and finally continuous light (LL) for the last 4 weeks. The particular Shift conditions aimed to simulate light/dark conditions of shift work on fast rotation program. As the dark phase was prolonged by 8 h every second day, it is the most common shift work. Further details on experimental design, technical and animal treatment can be found in [4].

Measured variables were recorded every 10 s, stored in PC by program A.R.T Gold 4.1 (DSI, St. Paul, Minnesota, USA). Permanent and wireless data acquisition enabled on-line monitoring of recording variables in order to control overall functioning of the recording system and animal conditions. For further data control and preprocessing manipulation Microsoft Excel and MatLab programs were used.

For spectral analysis modified Lomb-Scargle periodogram (LSP) [5] was adapted in MatLab environment (MatLab 2008b, MathWorks, Inc. USA). LSP approach is identical with estimation of harmonic contend of a data set at a given frequency by linear least-squares fitting to the model. This method has several advantages in comparison with traditional fitting techniques. The basic characteristic of our large data sets was the absence of regular sampling in recorded signal. Several reasons contributed to these irregularities: Manual weighting of the experimental animals, when wireless communication of the sensor with receiving plate was interrupted for a few minutes. Also occasional shorter malfunction of the recording system were involved. Still, our task was not to discard the data recorded for a number of days but rather to apply methods of analysis that are capable to deal with unevenly sampled data. Traditional spectral estimators need to modify irregularly sampled series by interpolation and even re-sampling. Unlike conventional Fourier analysis, LSP approach is capable to overcome dropout of smaller data fractions in time series. Another advantage of LSP approach is a possible choice of arbitrarily fine frequency step and examination of frequencies higher than the mean Nyquist frequency. Moreover, weighting of single power points occurs rather on a ‘per point’ basis instead of on a ‘per frequency interval’ basis. This means that the height of the peak is stable unlike in the case of Fourier types of analysis where height of a peak is confined with frequency step. As all other methods in the search for periodic signals, the LSP requires the assumption of independent Gaussian noise on the error term. Under this assumption one can estimate level of significance of resulting peaks. Peaks probability distribution is evaluated according to observance of certain amount of independent peaks. Basically, the LS algorithm is quite time demanding, however several less time consuming modifications with sufficiently exact approximation were developed, working at the level of N-logN computational time demand (N is a number of frequency components to be estimated).

3. Results and discussion

Spectra obtained by LSP approach reveal animal reactions to different regime conditions. In our analysis we focus on circadian (20-30 h) and ultradian (2-6) rhythms. Strength and period localization of power peaks in these ranges was changing (Fig.1) according to the conditions. While during LL conditions circadian contribution is highly suppressed, during Shift conditions 24 h peak is elongated to longer periods.

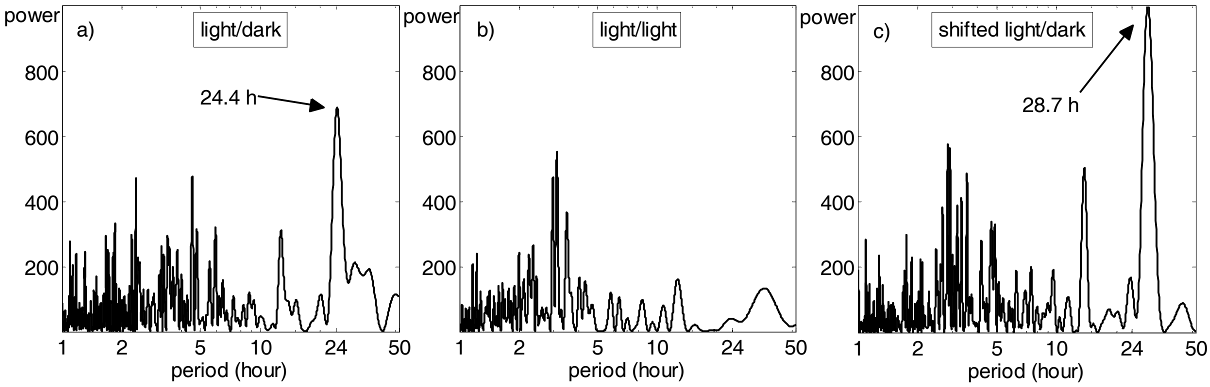

Fig. 1: Periodogram calculated by LSP approach for diastolic pressure of rat #1 for 3 different conditions: a) 12:12 light/dark revealing circadian peak near 24 h, b) persisting light conditions with emphasized activity of ultradian rhythms, and c) delayed shift in light/dark conditions with prolongation of circadian rhythm.

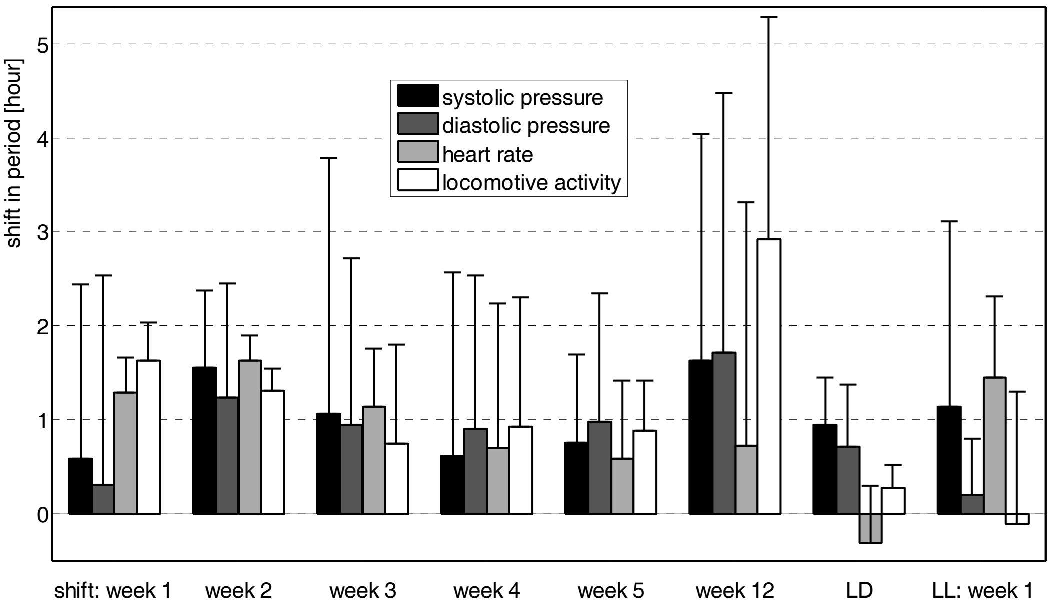

Fig. 2. Drift of main circadian periods from 24 h period represented by zero value on Y-axis. X-items represent week intervals under shift, light/dark and light/light regime. Mean data from all rats with standard deviations in a form of error bars are presented.

In Fig.2 the shift of circadian peak of 24 h is evaluated. The strongest drift occurred during the second and 12th week of the Shift conditions, suggesting more complex adaptation mechanisms of inner clocks. Initial wave of adaptation was weakened during the next weeks. Unfortunately, during week 6-11 no telemetry measurements were performed. Increased shift of SP and DP during LD conditions could be affected by tightly preceding of Shift conditions, while HR and LA seemed to be more flexible according to their reaction in this particular time point of the experiment. Whereas from the point of view of Fig.2 there were two vertexes in deviation of 24 h peak – in Shift week no. 2 and 12, in Fig.3 there is substantial difference between these 2 weeks. Relative amount of LSP power from circadian range in comparison to the amount of power in ultradian range was more strongly activated only in the first and second week of Shift regime. During the last two weeks of persistent light conditions circadian rhythms were relatively eliminated in the highest extend.

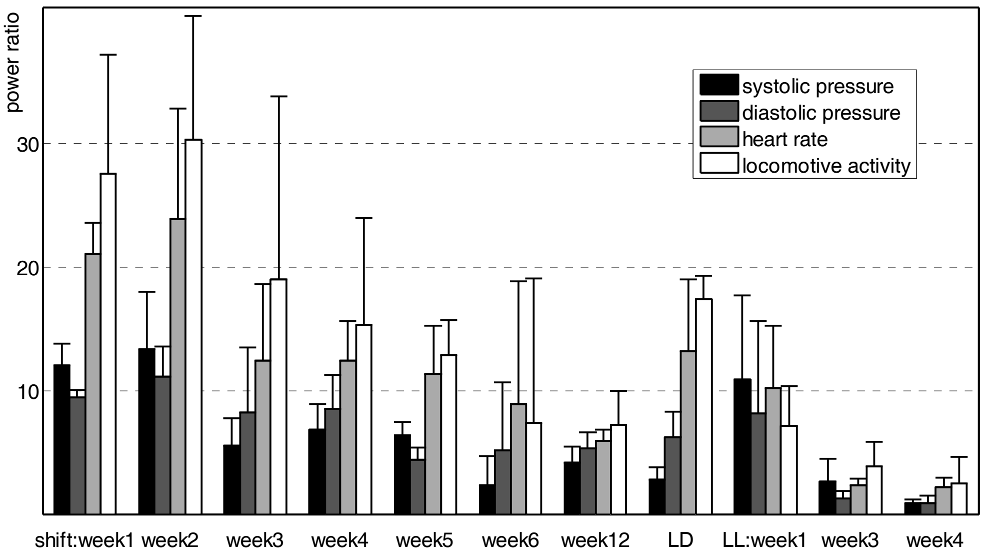

Fig. 3: Ratio of the power between circadian band (20-30 h) and ultradian band (2-6 h) during different regime conditions. X-items represent week intervals under shift, light/dark and light/light regime. Mean data from all rats with standard deviations in a form of error bars are presented.

We showed that the cardiovascular system of rats (blood pressure, heart rate) is able to adapt to delay shifts in light regime by lengthening of endogenous period of cardiovascular parameters. Moreover, ratio of circadian and ultradian cycles is changing. Results contribute to understanding of physiological changes that accompany shift work. In further work shifted regime in opposite – advanced direction will be spectrally analyzed.

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