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Characterization of the relationship between spontaneous locomotor activity and cardiovascular parameters in conscious freely moving rats

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Abstract

In freely behaving rats, variations in heart rate (HR) and blood pressure (BP) are coupled closely with changes in locomotor activity (Act). We have attempted to characterize this relationship mathematically. In 10- and 16-week-old rats, HR, BP and Act were recorded telemetrically every minute for 2 days under 12h:12h light-dark cycling. After examining data for individual rats, we found that the relationship between Act and HR could be approximated by the negative exponential function $HR(Act)=HR_{max}-(HR_{max}-HR_{min})*\exp(-Act/Act_e)$, where HR_{max}, HR_{min} and, Act_e are constants. These constants were calculated separately for light and dark periods by non-linear curve fitting. HR corresponding to maximal locomotion was similar during the light and dark phases, while HR at rest during the dark phase was higher than during the light phase. The range of HR variability associated with Act during the dark phase was similar in young and older animals, but minimal HR was significantly lower in older rats. The relationship between Act and BP was approximated with a similar function. We have found no differences between BP at rest and at maximal locomotion between light and dark and between 10-week and 16-week-old rats. Our results indicate that in rats, cardiovascular parameters are coupled to locomotion to a high degree; however both the HR and the BP reach maximal values when locomotor activity is relatively low. We also found that the phase of daily cycle affects HR in conscious rats independent of locomotor activity.

Keywords

Locomotor-cardiovascular coupling; heart rate; blood pressure; locomotor activity; telemetry; rat

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INTRODUCTION

Radiotelemetry provides a powerful non-invasive means for long-term monitoring of physiological parameters [1, 2]. Most often this technique has been employed to characterize rhythmic circadian and/or ultradian patterns in individual parameters for which a variety of methods have been employed, including autocorrelation, power spectrum analysis (Fourier transform) and cosinor analysis. However, much less attention has been directed to a particularly powerful feature of telemetry: the ability to assess and correlate two (or more) physiological parameters simultaneously.

A potentially significant relationship that has received limited attention is that between spontaneous locomotor activity and the primary cardiovascular endpoints of heart rate and blood pressure. Meinrath and D'Amato [3] and Schlatter et al. [4] reported high correlations between heart rate and locomotor activity. Waterhouse et al. [5] studied the sensitivity of blood pressure to locomotor activity where a linear relationship between these parameters was assumed. Lemmer et al. [6] applied a "purification" technique, which was developed earlier for circadian variability of body temperature, to dynamics of heart rate and blood pressure. In this study, the authors tried to recover "endogenous" circadian variations masked by activity using the proximity of the recovered endogenous curve to the cosine curve as a goal. One of the assumptions in such an approach is that the correction coefficients for locomotor activity are the same during dark and light periods [6, 7]. The conclusions of these studies suggest only that the heart rates of rodents are higher at night when locomotor activity is present [8] or involve untested assumptions about the relationship [5].

Thus, the relationship of spontaneous locomotor activity to cardiovascular endpoints has never been subjected to careful mathematical analysis that might permit the quantitative estimation of the contribution of locomotor activity to blood pressure and heart rate on a minute-to-minute basis with any degree of precision. Such an analysis would be of considerable interest from the perspective of basic physiology but might also prove to have considerable utility in factoring out effects on locomotion when assessing cardiovascular changes in a given experimental paradigm. For example, when examining the effects of an experimental antihypertensive agent that alter behavior, the contribution of observed changes in locomotion could be quantitatively assessed with regard to changes in blood pressure and heart rate.

To estimate the functional relationships between locomotion and cardiovascular parameters, we collected data on blood pressure, heart rate, and locomotor activity from chronically instrumented rats, then prepared and examined appropriate scatter plots of our data. All graphs clearly demonstrated a consistent but non-linear linkage between spontaneous activity and cardiovascular parameters. We then analyzed mathematically the relationship between two pairs of parameters – locomotor activity and heart rate, and locomotor activity and mean blood pressure. Using this information, we developed a technique to calculate locomotion-independent values of mean blood pressure and heart rate. Our newly developed approach was compared with a previously published technique to calculate heart rate and blood pressure associated with inactivity.

Either differences in the intensity of spontaneous locomotion or differences in mathematical parameters that determine behavioral-cardiovascular coupling [5] could ultimately affect cardiovascular parameters. Therefore, we tested our technique by analyzing and comparing datasets (1) from two periods that differed markedly with respect to levels of spontaneous locomotor activity day (light) vs. night (dark), and (2) from rats of different ages (10 weeks versus 16 weeks) which were serendipitously noted to have similar patterns and levels of locomotor activity but differed significantly with respect to cardiovascular status.

METHODS

Animals

Male Sprague-Dawley rats from three litters were used. Female rats were obtained from Harlan (Indianapolis, IN) at the 15th day of pregnancy and maintained under standard animal housing conditions with lights on 07-00 – 19-00. All deliveries occurred within 2 consecutive days. All litters were reduced to 11 pups on day 5 and weaned at the 21st day of age. Animals were housed at three animals per cage until the implantation of the telemetric probe. After the surgery, animals were housed singly. All procedures were in accordance with protocols approved by the Indiana University School of Medicine IACUC.

Telemetric probe implantation

Male rats were randomly selected for surgery from all three litters at 9 weeks (250–300 g) or 15 week (350–400 g). Rats were anesthetized with Nembutal (50 mg/kg, ip). The flexible catheter of the telemetric transmitter (PhysioTel® PA-C40 Small Animal Transmitter, Data Sciences Intl., St.Paul, MN) was secured surgically in the abdominal aorta with the tip just below the renal arteries. The transmitter was sutured to the abdominal wall. After the surgeries, the animals received an injection of buprenorphine (0.05 mg/kg, s.c.) and were monitored until recovery from anesthesia. Rats were housed in individual cages and allowed to recover from surgery for at least seven days to restore normal daily rhythms [9, 10].

Recording of telemetric data

Rats in their home cages (non-transparent cages with dimensions 40 × 20 × 20 cm) were placed over the receiver plates in a separate room. The rats were unrestrained and free to move within their cages with unlimited access to standard chow and tap water. Animals were left undisturbed for 2 days except for replenishment of food and water.

Hemodynamic data were sampled for 10 sec every minute and average heart rate, mean arterial blood pressure, and locomotor activity were stored. Rats were allowed to adapt to this new environment for at least 5 h. Recording started before 7 pm on the first day and finished after 7 am on the third day of recording. Therefore, the total duration of undisturbed continuous recording subjected to analysis was at least 36 h and included one complete 12-h light period and two complete 12-h dark periods.

For proper interpretation of data, it is important to understand how the telemetric system records various data. In the “scheduled sampling” mode, physiological parameters like blood pressure or heart rate are acquired only for a predefined period of time each cycle. They are

then averaged and recorded at the end of the acquisition period. For example, we had configured the system to record cardiovascular parameters each minute by averaging data obtained over 10 sec. This means that every minute, mean blood pressure and heart rate were recorded for 10 seconds (for example, from 0 to 10 sec) and stored at the end of the interval (at 10 sec). Unlike physiological parameters, locomotor activity is calculated from the intensity of the telemetric signal. The intensity changes depending on the distance of the probe from the receiving coils and the angle between the probe and the coils. The hardware follows the strength of the signal and generates an event when the strength changes for more than a threshold. The algorithm was designed in the following way: if the animal walks with a constant speed of 1 cm/sec for the entire minute, it is measured as 1 locomotor unit. Thus, according to this scale, 10 units is equivalent to 6 m/min, and 30 units to 18 m/min.

Monitoring signal strength, and as a consequence locomotor activity, is performed throughout the entire cycle (in our case 1 min). The software records the information about locomotion in the beginning of the monitoring periods. In the analysis module, new activity data appear at the beginning of the monitoring period, while cardiovascular data are added after the end of the monitoring interval. Therefore, in our dataset, an array of data for a specific cycle consisted of cardiovascular parameters measured between 0 to 10 seconds while activity was “counted” for the period from −60 to 0 sec. It is interesting that crosscorrelation between activity and heart rate is maximal at +1 min, while there is no shift between the activity and the blood pressure. Thus, despite the fact that activity recorded for a particular data point is on average collected 30 seconds before heart rate, it still takes an extra minute for the heart rate to respond to a change of locomotion.

Processing of the data

All data processing, calculations and fittings were performed in Microsoft Office Excel. First, the data from individual recordings was averaged for 60 min intervals to reveal daily rhythmicity. Then, original minute-by-minute data from individual recordings was analyzed for auto- and crosscorrelation using Statistica for Windows (Statsoft Inc., OK). Intervals of 640 minutes (from 7–40 to 6–20) from light and dark periods were used for analysis with lags from 0 to 240 min for autocorrelation and from -240 to 240 min for crosscorrelation. Data from the two dark periods were averaged.

Finally, minute-to-minute data from individual recordings was averaged for intervals of 10 min starting every 5 min (for example 5:00 – 5:09; 5:05 – 5:14; 5:10 – 5:19 etc). An abrupt change of lighting between day and night is an external stimulus, which affects the level of anxiety for up to 20 minutes [11]. Therefore, considering that our aim was to examine the correlation between spontaneous activity and cardiovascular parameters, the data around light-dark phase change (30 min before and after 7:00 am and 7:00 pm) were discarded. Averaged data were graphed using scatter plots (Act-HR; Act-MBP).

We used the Solver add-in module of Excel to fit the relationship between heart rate and locomotor activity for each individual recording with the following function (see fig.1 for a graphic presentation of constants employed)

$HR = HR_{max} - \Delta HR * EXP(-Act/Act_e)$, where

HR	– heart rate as function,
Act	– locomotor activity as an argument,
HR_{max}	– heart rate, corresponding to infinite locomotor activity (by extrapolation),
HR_{min}	– heart rate at rest corresponding to minimal locomotor activity,
ΔHR	– range of heart rate depending on locomotion, difference between HR_{max} and HR_{min},
Act_e	– coefficient, defining sensitivity of HR to locomotor activity.

Best fit was achieved by changing cells containing values for HR_{max}, ΔHR and Act_e to minimize the value of a target cell which contained sum of squares of differences between actual and calculated HR for all Activity-HR pairs. After obtaining best fit values for these three parameters, HR_{min} was calculated.

Four parameters (HR_{min}, HR_{max}, ΔHR, and Act_e) were calculated for each rat separately for light and dark periods, were analyzed separately and then compared. Because no differences were found between the parameters for the first and second night recordings, data for both dark phase intervals were pooled. The same calculations were performed for mean blood pressure (MBP) and locomotor activity data.

Frequency distributions were constructed for 10-week-old animals using a bin of 10 units for locomotor activity, 2 mm Hg for mean arterial pressure and 5 beats/min for heart rate. After the frequency distribution for locomotor activity was calculated for each illumination period, blood pressure and heart rate samples were separated on the basis of the activity level recorded during the same sample period. Samples associated with activity were defined as those for which the corresponding activity value was different from 0 and samples associated with inactivity were defined as those for which the corresponding activity level was equal to 0. Frequency distributions for heart rate and blood pressure were constructed separately for samples associated with activity and inactivity. Frequency values for the two dark phases recorded in individual animals were averaged. The modes of each distribution were determined. HR_{min} was determined as a maximum of lower mode of HR associated with inactivity; HR_{max} as maximum of higher mode of HR associated with activity. Minimal and maximal BP were determined similarly. Coefficients of correlations were calculated between minimal, maximal and range of HR and BP obtained by analysis of frequency distribution and the same parameters obtained the by negative exponential fitting technique.

Statistics

All continuously recorded variables (HR, MBP, Act) were evaluated by using one-way (age) ANOVA for multiple comparisons with Fisher's LSD test when ANOVA indicated significant differences between groups.

In exponential approximations, calculated data for individual animals were excluded from statistical analysis if approximation was close to being linear (exponential coefficient $Act_e > 50$ paralleled with physiologically nonsense values of $HR_{max} > 700$ or $BP_{max} > 160$). If data

were excluded for any period, all data for this animal were excluded from statistical analysis. Comparisons of calculated parameters between age groups were made by one-way (age) ANOVA for multiple comparisons (day vs night) followed by Fisher's LSD test when ANOVA indicated significant differences between groups.

A value of $P<0.05$ was considered to indicate a significant effect in all comparisons.

RESULTS

Circadian and ultradian variability of cardiovascular parameters and locomotor activity

Both cardiovascular parameters (heart rate and mean blood pressure) as well as locomotor activity showed clear circadian changes in all rats. Average heart rate during the dark period was 50–75 beats/min higher than during the light period in both groups of animals, and mean blood pressure was also higher by 10 mm Hg during the dark period (Fig.2).

Older rats had significantly lower heart rates, while blood pressure did not change with age (for age $F(1,10)=1.391$; $P=0.266$). There was also no difference in spontaneous locomotor activity between the two age groups (for age $F(1,10)=0.00443$; $p=0.948$). Locomotor activity was near zero during the light photoperiod with only occasional spikes of activity that occurred randomly in different animals. A histogram of locomotor activity for dark and light phases (Fig. 3) had an exponential shape. The distribution of frequencies of locomotor activity was dominated by inactivity during both light and dark phase. The percent of time animals spent at rest was close to 90% during the light phase and above 50% during the dark phase. However, all levels of activity were more likely to occur in rats at night. Maximal observed locomotor values were above 20 units/min, but they represented a minimal percentage of all data points.

Typical recordings from a 10-week-old animal are shown as Fig.4. A coincidence of spikes of spontaneous activity with increases of HR and BP was evident in all individual recordings. The presence of cycles in Fig.4 is not a representation of any reproducible rhythms. If rhythms had been present, evidence of them would have been apparent on the autocorrelations and/or results of the Fourier transformations, and as shown below, it was not.

In order to estimate the relationship between HR and Activity and BP and Activity, corresponding scatter plots were prepared. Results of a typical experiment (same animal as used for Fig.4) are presented at Fig.5. Both HR and BP increased with increasing locomotion. However, minute-by-minute data (closed small circles) demonstrate a clear maximum for each parameter, which was reached at relatively low levels of activity. Averaging over ten minutes (Fig.5, open triangles) makes the exponential relationship clearly visible. Rats did not exhibit sustained intense locomotion over prolonged periods of time, so averaging also removes a high activity component from the curve.

There were no significant autocorrelations of any studied parameter with lags exceeding 20 min. Fourier transformation using 640 min intervals of data (day and night were analyzed separately) did not reveal any rhythmic patterns within either illumination period (data not

shown). For both light and dark periods, all autocorrelation and crosscorrelation coefficients are significantly different from zero at all lags less than 15 min. Maximal crosscorrelation between activity and heart rate was found at the lag of +1 min, while crosscorrelation between activity and blood pressure was maximal at zero lag.

Locomotor activity – cardiovascular coupling

The results of exponential approximation of Act-HR and Act-BP dependences are shown in Table 1. In older rats, both minimal and maximal HR were lower than in younger animals. Minimal heart rate during the light photoperiod was lower than during the dark photoperiod. Maximal heart rate was similar during light and dark which resulted in a lower range of activity-dependent HR during the dark photoperiod. The sensitivity of heart rate to locomotor activity (characterized by Act_e) was similar in younger and older animals.

Frequency distribution of heart rate and blood pressure values

Frequency distributions show the frequency with which values of the parameters were observed. This analysis was performed separately for light and dark phases for data obtained in 10-week-old rats. Values of heart rate sampled during the dark phase were distributed bimodally (Fig.6). After frequency distribution was calculated for the complete set of data, each data set was divided into one associated with activity and another associated with inactivity as described in [12]. This permitted the identification of the origin of each maximum in the frequency distribution. The lower mode of heart rate values in the complete dataset appeared to be associated primarily with inactivity. Heart rate values associated with activity appeared almost exclusively in the higher mode. The higher mode was nearly absent in the frequency distribution for heart rate values sampled during the light period. Similar trends were apparent for the frequency distribution of blood pressure values (Fig.7).

To compare results of frequency distribution analysis with the results of proposed exponential fitting, the values of minimal and maximal HR and BP calculated from exponential fitting are shown with arrows on both Figs. 6 and 7. Maxima for distribution of HR and BP associated with activity and inactivity are shown in Table 2.

The higher mode of heart rate was not different for light and dark phases. The lower mode of heart rate distribution for the dark phase was significantly higher than the lower mode for the light phase. Comparison with the results of the exponential fitting technique showed that the maximum of higher modes matched the maximal heart rate, while the maximum of the lower mode was not different from minimal HR. For most parameters, correlation coefficients between parameters obtained by the two techniques were above 0.8 (Table 2).

Mean blood pressure values associated with activity and inactivity were not different between light and dark phases, similar to the results of the exponential fitting procedure.

DISCUSSION

Physiological parameters are dependent on many variables. In “purification” techniques to obtain locomotor-independent physiological parameters, “non-activity masking effects” accounted for an insignificant component of circadian variability of body temperature [13].

Our findings point to a strong association between cardiovascular parameters and spontaneous locomotor activity quantitated by telemetry in undisturbed freely moving rats. Unlike body temperature, both heart rate and blood pressure demonstrate high frequency changes, which could be smoothed by averaging. Fourier transformation using 640 min intervals of data did not reveal any rhythmic patterns within either illumination period (data not shown). To estimate an interval appropriate for averaging data for the exponential fitting procedure, we used auto- and crosscorrelation analysis. Data points that are close to each other in time are more highly correlated than more distant ones, unless there is a periodicity. Therefore, autocorrelation analysis is used to reveal the periodicity, but also shows how "inert" a parameter is or how fast the variations of specified parameters occur.

In our study, there were no significant autocorrelations with lags exceeding 20 min. In all groups (both age groups and both illumination periods) there were significant autocorrelations and crosscorrelations for all three parameters at intervals up to 15 min. At the same time, we found that averaging over 10 min eliminates most of the "high-frequency" variability, so this interval was selected.

The proposed analysis does not depend on the presence or absence of any rhythmicity but requires the presence of variability. Considering that the periods of short-term oscillations of parameters are significantly less than 24 hours, they represent ultradian variability.

Physiological meaning of calculated parameters

There are three major physiological parameters obtained as a result of the exponential fitting of circadian data. First, we were able to extrapolate heart rate at rest (HR_{min}). During the dark phase, because some rats were constantly moving, mathematical extrapolation was the only way to obtain heart rate and blood pressure "at rest". The way we obtained this parameter was by using an essentially ANCOVA-negative exponential model, a "purification" technique, used earlier for analysis of body temperature in Mongolian gerbils [13]. Younger rats (10-week-old) had higher heart rates than older (16-week-old) animals. These differences in heart rate represent differences between adult rats of different ages, because rats reach puberty at 4–5 weeks of age, while the average lifespan in Sprague-Dawley rats exceeds 2 years (104 weeks). The decrease of 20–30 beats/min in resting HR develops over a course of 6 weeks and should be considered in longitudinal studies, because it may mask or exaggerate effects of drugs or physiological responses.

Second, Act_e provides an estimation of the sensitivity of the cardiovascular system to locomotor activity. It was surprising that in our study Act_e appeared to be below 2 units while locomotor activity could be as high as 30–40 units. This value of Act_e means that when locomotor activity is 4 units, heart rate is within the top 5–10% of its range. The manufacturer of the telemetry system employed (DSI, St.Paul, MN) calibrated the locomotion recording so that 1 unit corresponds to the speed of 1 cm/sec. Thus, 4 units represented a speed of 2.4 m/min. For comparison, healthy Sprague-Dawley rats with weights 300–400 g are able to run on a treadmill at 18 m/min for more than an hour (Zaretsky DV, personal observations).

The fact that virtually any locomotor activity results in submaximal or maximal tachycardia has been demonstrated in other species. In baboons, most activities (standing vertical, walking, jumping, running) were characterized by similar tachycardia (137–147 beats/min) which was different from heart rates during lying or sitting (107–111 beats/min) [14]. A similar observation was made in rhesus monkeys [15]: sitting with minimal movements resulted in half-maximal increases in heart rate compared with sitting still, while standing still evoked maximal tachycardia. Locomotion did not increase heart rate above values observed when animals were standing motionless. The quick "saturation" of HR and BP with low levels of locomotor activity prohibits the use of the slope of Act-HR dependence without limiting the range of acceptable activity to express the sensitivity of the cardiovascular system to locomotor activation as it was done previously [5]. We hypothesize that the principle of "all or none" is typical for locomotor-cardiovascular coupling in mammals, while this study is the first experimental demonstration of it in rodents.

Third, we have found that there is a maximal value of heart rate and blood pressure associated with spontaneous locomotion. Saturation was obvious to the eye in "activity-heart rate" plots for every rat in this study, despite "biological noise" that resulted in a linear fit in a few animals. In contrast to our findings, in the study of Lemmer et al. [6], the masking component of heart rate (component of heart rate depending on locomotion, "purified" by the study) was almost linearly proportional to locomotor activity and no trend for saturation was evident. This discrepancy could be due to several differences in our approach. First, Lemmer et al [6] recorded total activity over an entire 15 min period, while cardiovascular parameters were recorded only at the end of this interval. Crosscorrelations are barely significant at 15 min, so intense locomotion at the beginning of a 15-min interval sufficient to result in high average locomotion for the period would not necessarily be accompanied with high HR at the end of the period. Conversely, if mild locomotion occurred only at the very end of the interval, the average locomotion for the period may still be low while the corresponding HR for this interval will be high (due to the rapid saturation of the effect on HR with intensity of the locomotion). However, since crosscorrelations are significant at lags less than 15 min, there should be a trend to higher HR at higher locomotion using this interval. We have re-plotted some of our data using activity averaged for 15 min, while corresponding HR was taken at the end of the corresponding interval (for example, locomotor activity was averaged for the interval 5:00 to 5:14, while HR was taken at 5:14). The resulting scatter plot appeared to be more linear with a smaller trend to saturate. Second, Lemmer et al [6] reported a value they termed a "masking factor", calculated to best fit with the assumed cosine shape of the "endogenous" (independent of locomotion) rhythm. Our technique did not employ this assumption. In a later study from the same group [7], evidence of the saturation effect so prominent in our data was again not evident. This latter study used minute-by-minute data, but it again employed a "masking factor", and not raw data. Both their studies [6, 7] also rely upon the hidden assumption that the effect of activity on cardiovascular parameters could be described by a uniform function across the circadian cycle. Our results contradict such an assumption.

The saturation phenomenon means that HR does not exceed a maximum solely due to locomotor activity. The most surprising finding was that this value is relatively low, i.e., 410–450 beats/min depending on the age of the animal. This was much less than maximal

HR developed in response to experimental emotional stress (500–510 beats/min, [16]), while in conscious rats chemical stimulation of the hypothalamus with kainic acid could evoke a tachycardia up to 560–580 beats/min (DV Zaretsky, unpublished observations). Cardiovascular changes associated with spontaneous locomotor activity resemble those during exercise, especially voluntary exercise. Indeed, Yancey and Overton [17] found that 250 g male Sprague-Dawley rats (same strain from the same source and of the same age, as in our 10-week-old group) had baseline heart rates of 350±10 beats/minute recorded telemetrically (minimal value for day-time in our study is 347 beats/min). Tachycardia during voluntary exercise was 428±14 beats/min [17], which is very close to the heart rate corresponding to maximum activity observed by us (438±11 beats/min). However, the same rats when forced to locomote on the treadmill exhibited higher rates of 470±12 beats/min [17], which is closer to values of stress-induced tachycardia. Thus, forced exercise may be seen as a stressor that can thus result in higher heart rates than those resulting from voluntary exercise. Stress has been reported to mask heart rate responses to workload in mice as well [18].

Minimal and maximal HR at day and night

Maximal HR did not differ between dark and light phases (Table 1). It is not surprising, as maximal cardioacceleratory drive is associated with maximal locomotor activity and so occurs when the animal is awake regardless of the phase of illumination. However, minimal HR during the dark phase is higher than that during the light phase. Therefore, the range of HR dependent on locomotor activity during the dark phase is smaller than the range during the light phase. Because rats are nocturnal, the transition from minimal movement to inactivity during the dark phase, on average, corresponds only to the cessation of movement, while during the light phase animals usually fall asleep. Transition to sleep could explain the lower minimal HR values during the light phase [19].

However, if the differences in minimal HR between daytime and night will be explained only by more frequent transition to sleep during the day, then the curves at non-zero locomotion will be same. In that case only zero-locomotion data points will be lower. If so, the exponential fitting will result in higher Act_e values. We could not find the support for this in our data. Therefore, we conclude that circadian input affects heart rate independent of locomotion, and most likely can not be explained by the higher percentage of time the animal spends sleeping during the day. Such a conclusion is important because day-night variability in locomotor activity and heart rate or blood pressure were used previously for estimation of locomotion-cardiovascular coupling [20]. Meinrath and D'Amato [3] showed that variability of heart rate has a component which is independent of activity. In their study since there was no separate analysis of phases of illumination, differences in minimal heart rate between phases of light cycle could be one of the components providing the independence apparent in their data.

Studies of the neuronal pathways responsible for daily rhythmicity have demonstrated a key role for suprachiasmatic nucleus (SCN). Scheer et al. [21] demonstrated a loss of daily variability of heart rate after lesions of the SCN and also showed that a light stimulus evoked a reduction in HR in intact but not in SCN-lesioned rats. Our finding of lower heart

rate during light phase independent of activity is consistent with the bradycardic action of illumination in intact rats.

Comparison of exponential fitting with frequency distribution approach

Minimal (independent of locomotion) and maximal (dependent of locomotion) heart rate and mean blood pressure in conscious rodents can be determined by an analysis of frequency distributions of these parameters [12]. In mice the distribution of those frequencies has an obvious bimodal shape, similar to what we have found in our data. Values of higher and lower modes for our population of animals determined by the analysis of frequency distribution (Table 2) were not only similar to the results of the exponential curve-fitting procedure (Table 1), but also exhibited strong correlation. We conclude that both techniques could be used to separate the effects of locomotion on cardiovascular parameters.

However, the approximation using an exponential function has some major advantages over the frequency distribution approach. First, during day-time the data associated with activity has a low number of observations. Increasing the time of observation is unlikely to improve the reliability of the method because the curve lacks a well-defined mode. Unlike the histogram, in the fitting procedure an increased recording length is more likely to provide sufficient data for improved curve-fitting. Also, fitting with the exponential function permits the analysis of the shape of dependence between locomotor activity and heart rate or blood pressure by finding characteristic Act_e. While we did not find this parameter changed in any of our experimental groups, we expect that it could be different in some situations [5].

To conclude, we have developed and tested the technique of analysis of locomotion-cardiovascular coupling using an exponential fitting procedure. The procedure permits the determination of the values of cardiovascular parameters independent of locomotion as well as the range of heart rate and blood pressure associated with spontaneous locomotor activity. Our findings in rats support observations in other mammals that relatively low levels of locomotor activity are accompanied by maximal tachycardia and increases of blood pressure induced by spontaneous motor activity. Thus, changes in heart rate and blood pressure that are associated with spontaneous locomotor activity are relatively limited to a narrow range of relatively low values.

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Highlights

The relationship between spontaneous locomotor activity and heart rate as well as between locomotor activity and mean blood pressure in freely moving rats could be approximated by the negative exponential functions.

Both the heart rate and mean blood pressure reach maximal values when locomotor activity is relatively low.

The phase of daily cycle affects heart rate in conscious rats independent of locomotor activity.

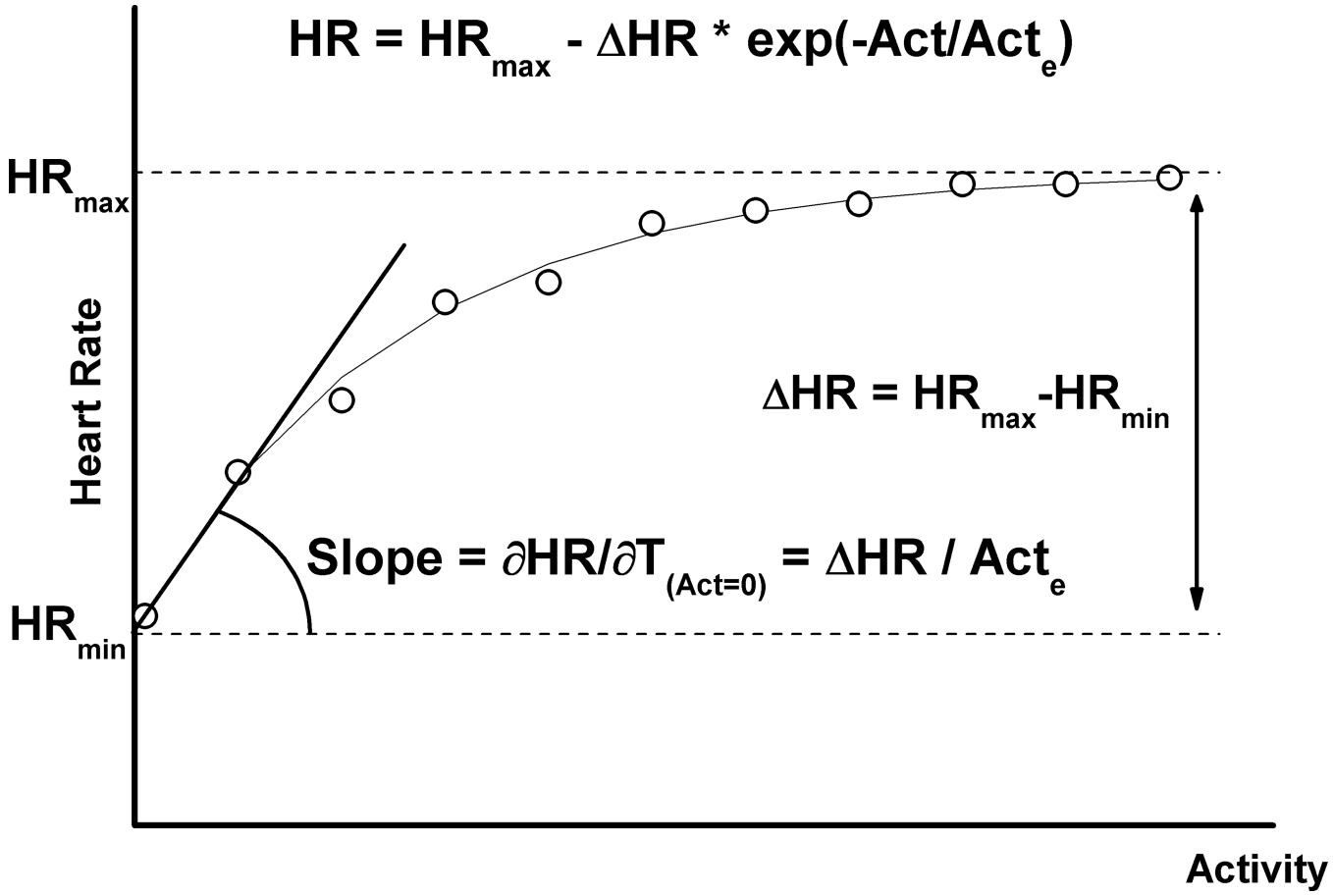

Fig.1.

Graphic representation of parameters used in the fitting exponential function describing dependence of cardiovascular parameters from locomotor activity. Open circles – model exponential curve with added noise.

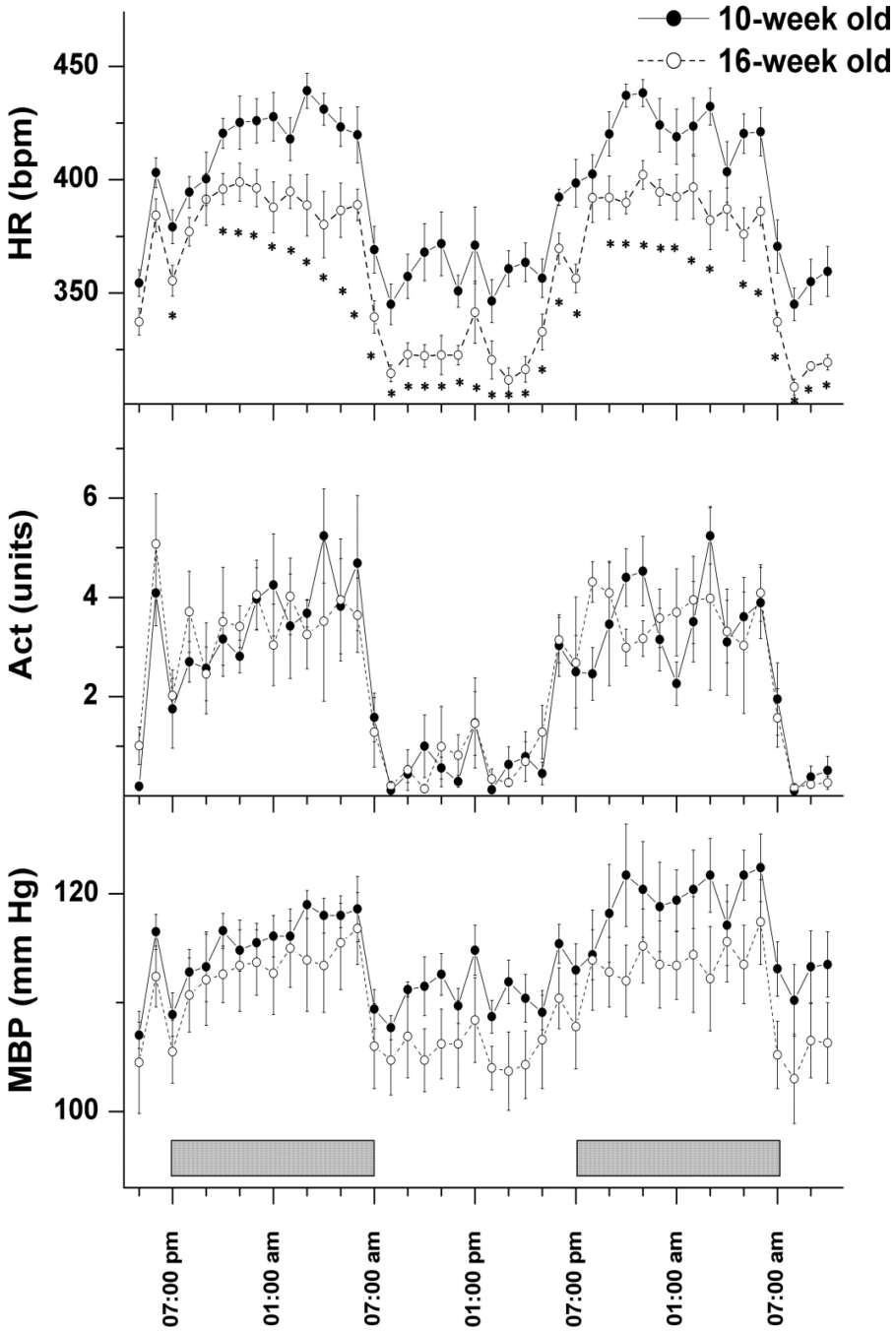

Fig.2.

Circadian variations of heart rate (HR), locomotor activity (Act) and mean blood pressure (MBP) in 10-week and 16-week old rats (mean±SEM, N=6 rats/group). Dark phase is marked by gray rectangles. * - significant differences between groups ($p<0.05$).

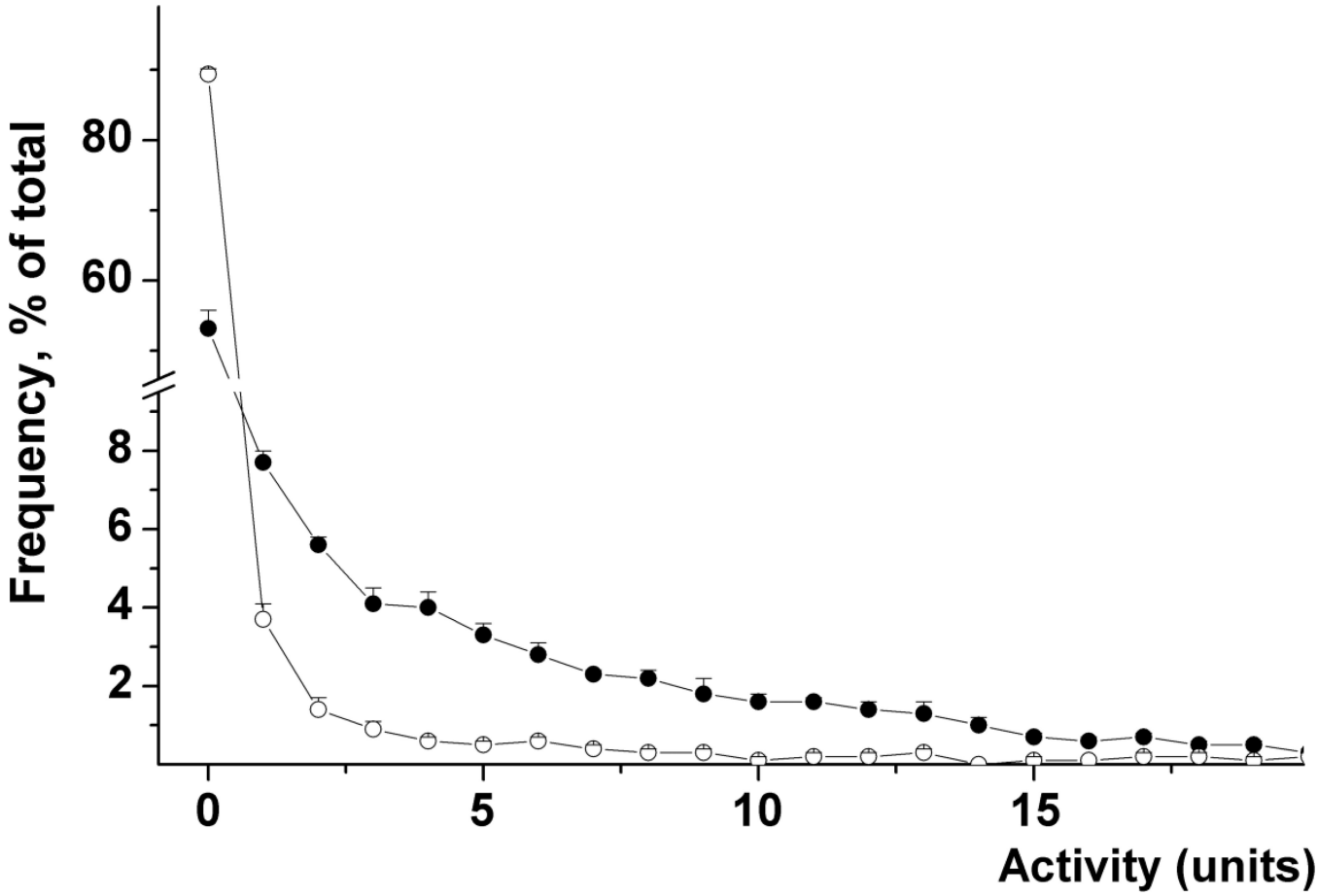

Fig.3.

Histogram of distribution of locomotor activity during light and dark photoperiods in 10-weekold animals. Filled circles – dark period; open circles – light period.

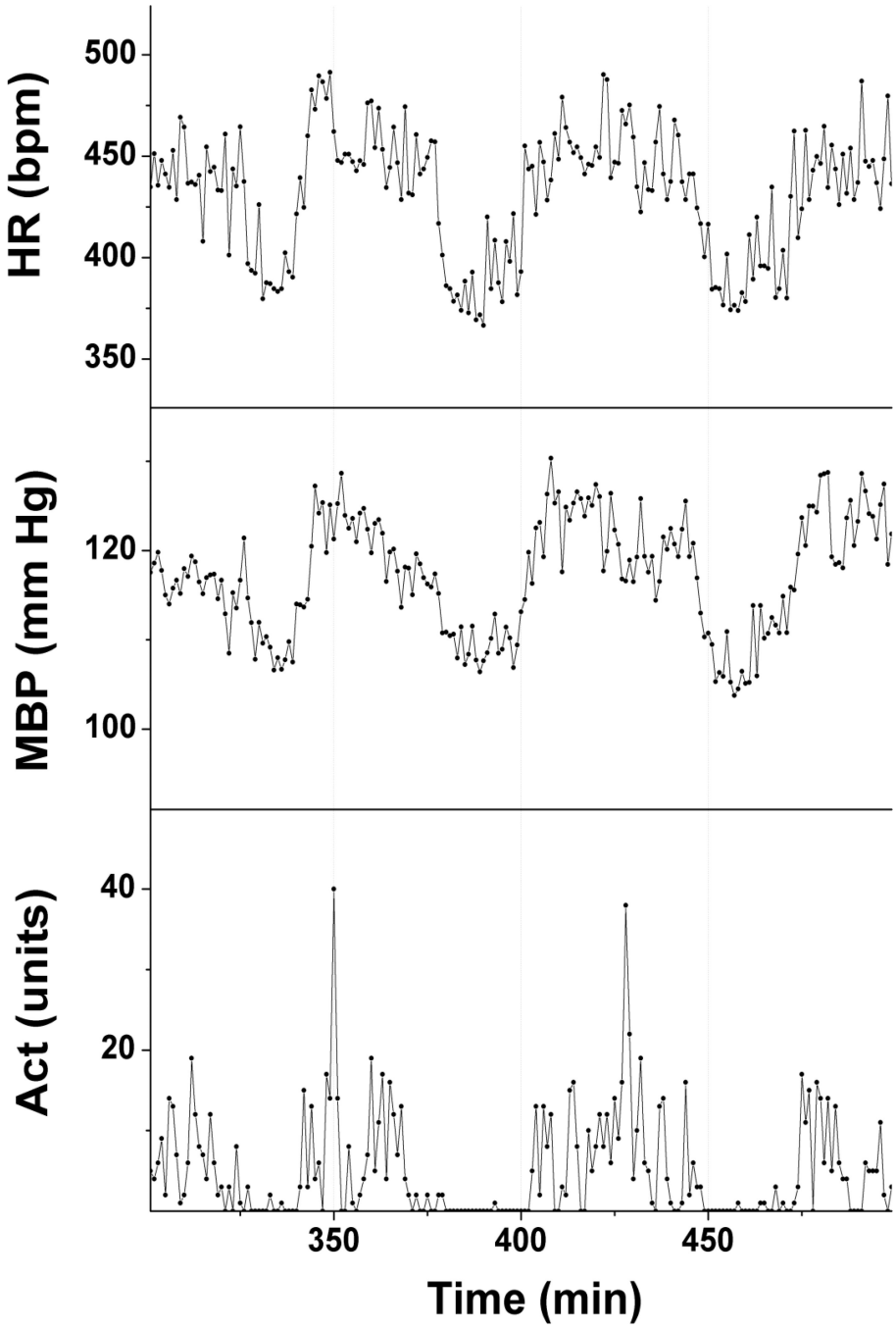

Fig.4.

Original 3 hour-long minute-by-minute recording of heart rate (beats/min), blood pressure (mm Hg) and locomotor activity (units) from dark phase in a typical 10-week-old rat.

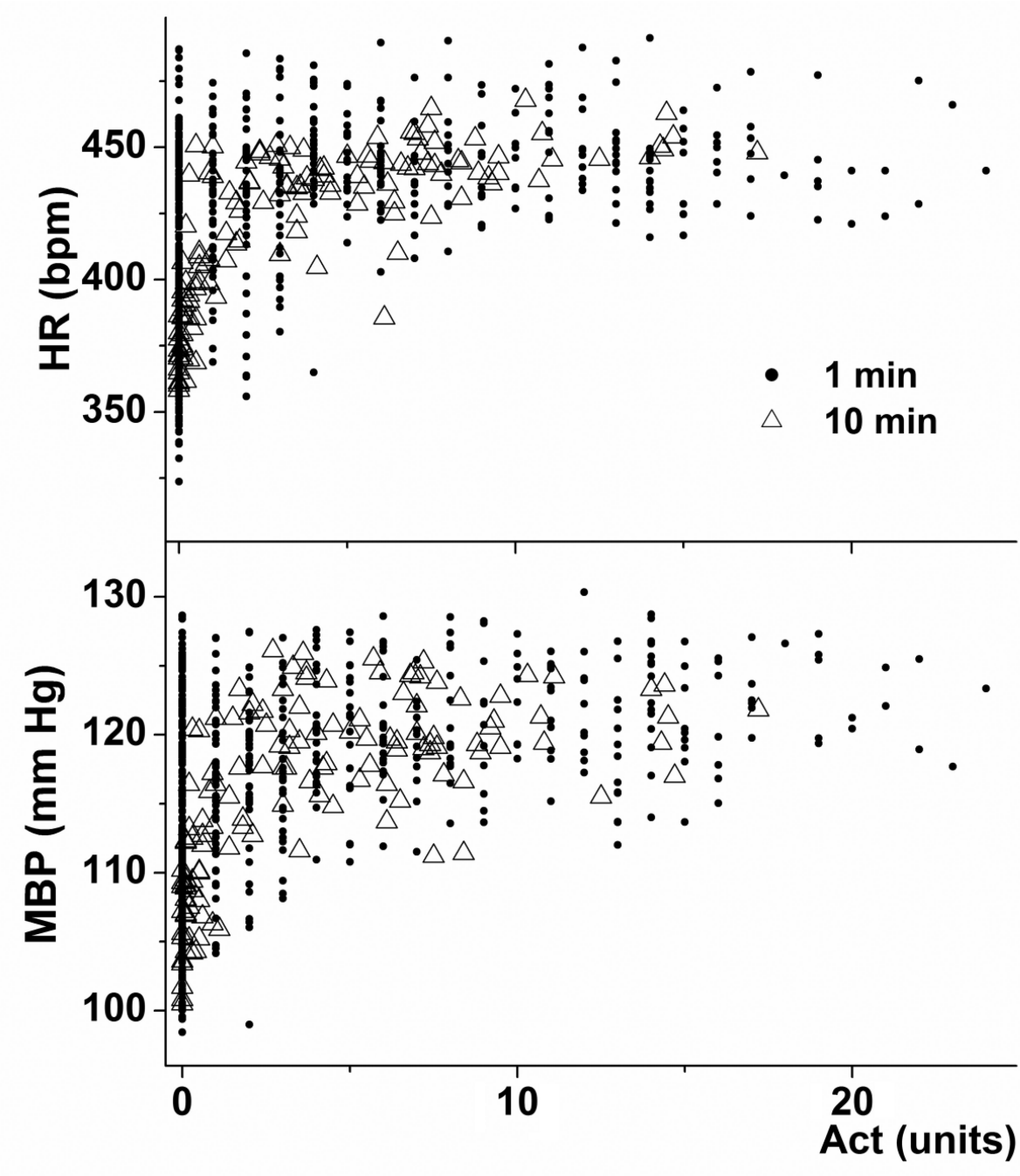

Fig.5.

Dependence between activity, heart rate and blood pressure in results of typical experiment of a 10-week-old rat. Results of averaging data over different intervals are shown: small dots – original data (1 min); open triangles – averaging by 10 min.

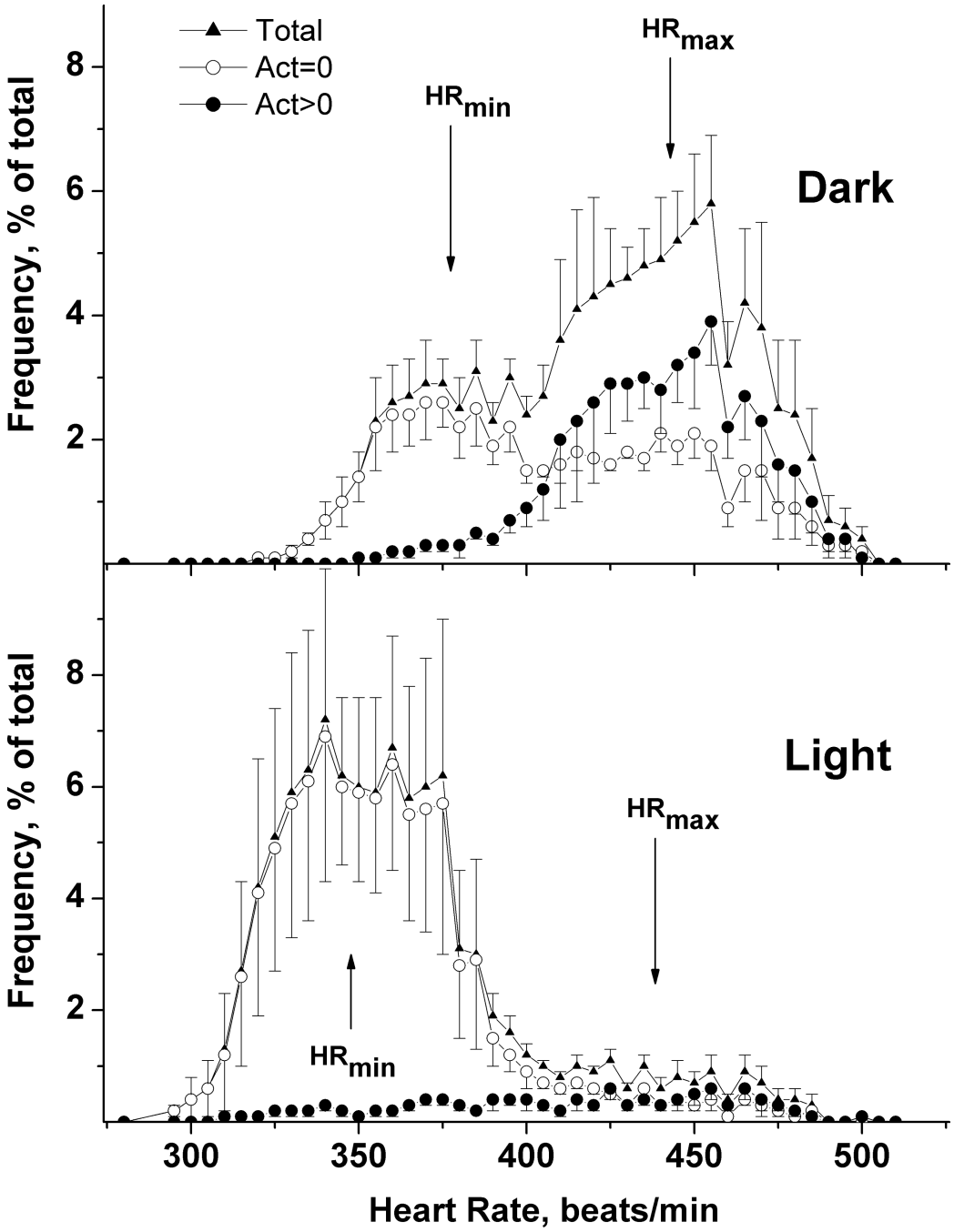

Fig.6.

Histogram of frequency distribution of heart rate during dark and light phases (triangles) and components of this histogram representing distribution with data points associated with inactivity (open circles) and with activity (closed circles). HR_{min} and HR_{max} are parameters calculated by negative exponential fitting technique.

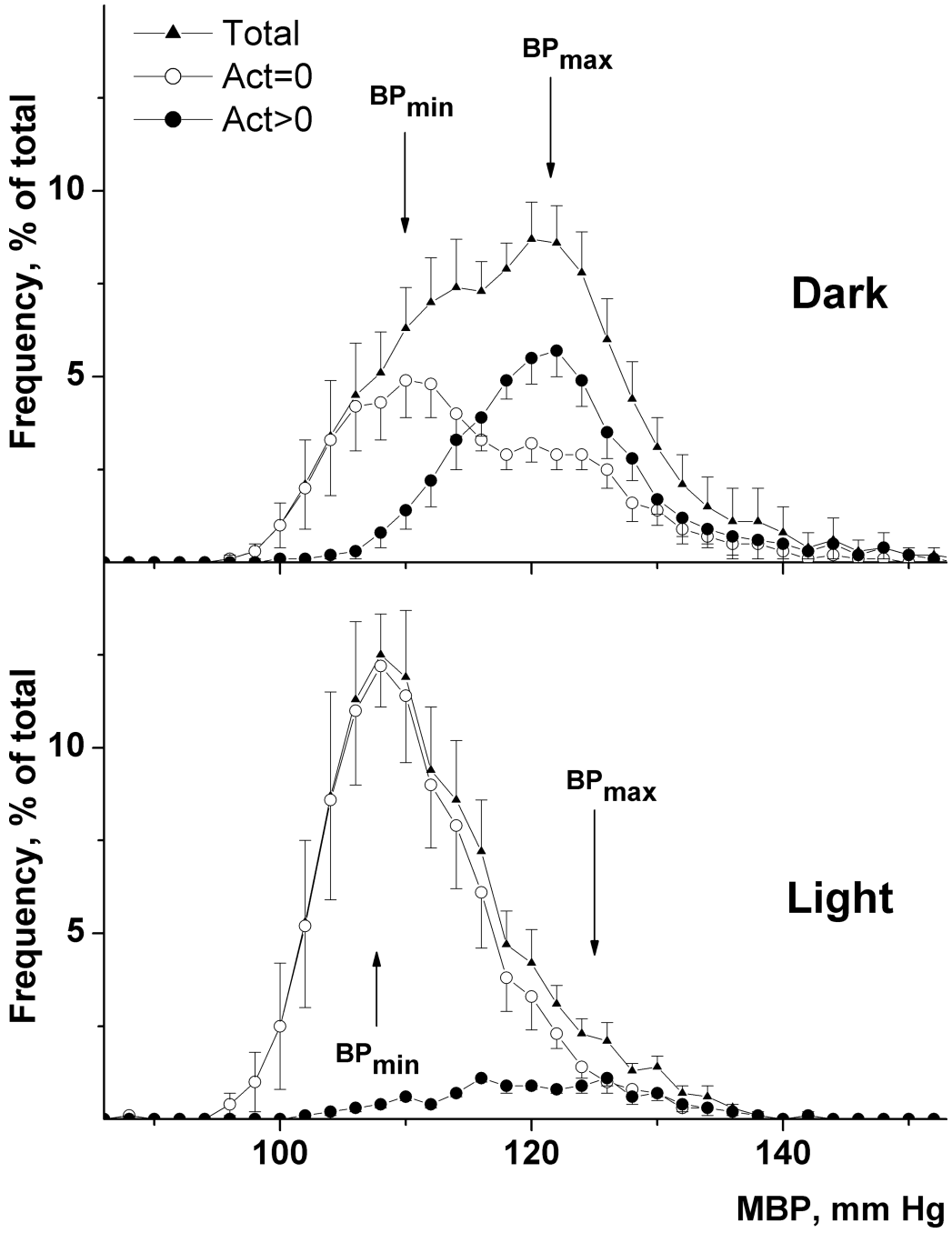

Fig.7.

Histogram of frequency distribution of mean blood pressure. Symbols are as described in Fig.6. BP_{min} and BP_{max} are parameters calculated by negative exponential fitting technique.

Table 1

Parameters of Act-HR and Act-MBP relationships calculated by fitting with negative exponential function in both age groups for light and dark phases.

Parameter	10-week		16-week	
	Light	Dark	Light	Dark
Activity – Heart rate				
	N=6	N=6	N=4	N=4
HR_{min} (bmp)	347±8	377±6 *	314±3 #	355±7 *#
HR_{max} (bpm)	438±11	442±6	408±8 #	410±13 #
ΔHR (bpm)	91±5	64±3 *	95±8	56±7 *
Acte (units)	2.0±0.2	1.4±0.3	2.6±0.3	1.5±0.3
Activity – Blood Pressure				
	N=6	N=6	N=4	N=4
BP_{min} (mm Hg)	108±1	110±3	105±4	107±4
BP_{max} (mm Hg)	125±3	121±2	124±5	120±5
ΔBP (mm Hg)	17±2	11±1 *	18±2	12±2 *
Act_e (units)	2.0±0.8	1.5±0.4 *	2.5±0.3	1.2±0.3

* - significant difference vs light phase;

- significant difference vs value for corresponding phase in 10-week-old group.

Table 2

Minimum and maximum HR and BP obtained by analysis of frequency distribution and their coefficients of correlation with same parameters obtained by fitting with negative exponential function.

Parameter	10-week-old (N=6)	
	Light	Dark
HR_{min} (beats/min)	347±8 (R=0.996)	372±6 * (R=0.919)
HRmax (beats/min)	434±14 (R=0.928)	445±7 (R=0.897)
BP_{min} (mm Hg)	108±1 (R=0.958)	109±1 (R=0.578)
BP_{max} (mm Hg)	122±2 (R=0.661)	120±1 (R=0.882)

* - significant difference vs light phase;