Spontaneous short-term fluctuations in the daily pattern of heart rate, body temperature and locomotor activity in the laboratory rat

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Summary

Heart rate (HR), body temperature (BT) and locomotor activity (LA) were measured continuously over 5 days in freely moving rats. In addition to the well-known circadian rhythms, all variables exhibited considerable fluctuations in amplitude mainly during the dark, but also in the light periods. The values of HR varied from 286 ± 12 to 470 ± 26 b.p.m. and BT from 36·15 ± 0·15°C to 38·45 ± 0·25°C. The large variability of HR, BT and LA within a single day was due more to large short-term fluctuations within periods of about 3–5 hours duration, than to differences between the light and the dark period. Good consistency of daily patterns and similarity of the 3 variables was found within the animals. Usually there were 3 or 4 regular peaks during the dark and often another peak 3–4 hours after the onset of light. Correlation coefficients, calculated on the basis of 5-min mean values, were highly significant ($P<0·001$) for LA vs HR (0·61–0·73), LA vs BT (0·40–0·53), and HR vs BT (0·61–0·68). Between-hour correlations were higher than these common correlations of 5-min values. HR vs BT (0·76–0·83) and LA vs BT (0·63–0·79) correlated as well as LA vs HR (0·72–0·83). The short-term fluctuations (within-hours) gave lower correlation coefficients for LA vs BT (0·23–0·32) and HR vs BT (0·29–0·41) than LA vs HR (0·40–0·70). This seems to result from a physiological delay of BT relative to HR and LA.

There is evidence to suggest that many physiological functions in the rat have periodic fluctuations lasting only a few hours, in addition to the well-known circadian rhythms. These short-term fluctuations were observed in locomotor activity (LA) (Honma & Hiroshige, 1978*a,b,c*; Lemmer, Caspari-Irving & Weimer, 1981; del Pozo, 1978), body temperature (BT) (Honma & Hiroshige, 1978*a,b,c*; Miles, 1962), heart rate (HR) (Büttner & Plonait, 1980), and endocrine functions (Tannenbaum & Martin, 1976; Tannenbaum, Rorstad & Brazeau, 1979). Although simultaneous determinations of BT and LA (Abrams & Hammel, 1965; Bolles, Duncan, Grossen & Matter, 1968; Honma & Hiroshige, 1978*a,b,c*) or LA and oxygen consumption (Bramante, 1961; Morrison, 1968) in the rat confirmed the close connection between these variables, little information is available on simultaneous variation of HR, BT, and LA in the freely moving rat (Meinrath & D'Amato, 1979). The main reason for this lack of information seems to be that simultaneous long-term measurements of HR and BT using methods such as recordings *via* light cables result in disturbance of the animals.

The purpose of this study was first a detailed examination of the daily patterns of HR, BT, and LA, and secondly an analysis of the interrelationship among these three variables. To avoid the difficulty mentioned above, HR and BT were measured by a peritoneally implanted telemetric system in unrestrained animals.

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Materials and methods

Animals and housing

Male LEW/Ztm rats about 100–140 days old were used. Their average weight during testing was 346 g. The animals were kept in an animal room under controlled conditions (room temperature 22 ± 1°C; relative humidity 55 ± 5%; light period from 6 am to 6 pm local time; light intensity on the level of the cages 300 lux). After implantation of telemetry transmitters the animals were kept individually in polycarbonate cages ('Makrolon' type III 55 X 33 X 20 cm) on sterile wooden granules. Pelleted diet (Altromin 1320) and tap water from Makrolon bottles were offered *ad libitum.* The animals were free from all pathogens specified in the GV-SOLAS list (GV-SOLAS, 1977).

Measurement of HR, BT, and LA

Telemetry transmitters weighing less than 5 g were used to measure HR and BT. They were implanted in the intraperitoneal cavity, with two electrodes of Teflon insulated V2A steel cord (Medwire Corp. 316SS7/44T) leading subcutaneously to the thorax (Plonait & Büttner, in preparation). Power saving pulse code modulation allowed continuous transmission over a period of 3–5 weeks unless trouble occurred with the electrodes. The transmitted ultrashort radio waves were detected by a nearby receiver and decoded by a demodulator. After demodulation, filtering, and amplifying the ECG was converted to HR by an HR-meter. Implantation of the telemetry transmitters took place under anaesthesia 9–12 days before testing. This time was sufficient for the animals to recover (Büttner & Plonait, 1980).

LA was measured by a capacitance system (Plonait & Büttner, in preparation) which gives a signal amplitude nearly proportional to the horizontal movement of the animal. HR, BT, and LA was recorded simultaneously at 10 sec intervals by a microcomputer. These measurements were used to calculate mean values over 5 min. All further calculations were based on these 5 min averages. Parallel paper recordings allowed a continuous control of HR, BT, and LA.

Results

Example for short-term fluctuations

Fig. 1 illustrates the fluctuations of BT, HR, and LA measured continuously and simultaneously in a single rat (No. 3) over a period of 3 days under a LD cycle of 12:12 h. The rapid fluctuations of the variables, especially HR and LA made it necessary to smooth the 5-min mean values obtained from the analysis. This was done using a recursive lowpass digital filter (Bendat & Piersol, 1971) which works similarly to exponential smoothing, but allows definition of a distinct cut-off frequency. The 5-min values were smoothed in two different forms, firstly by a cut-off frequency of 40 min (upper line) to remove strong fluctuations of less than 40 min duration, and secondly by a cut-off frequency of 24 h (lower line) to suppress fluctuations of less than 24 h.

Filtration with a cut-off frequency of 24 h shows distinct circadian fluctuations of BT and HR with an approximately sinusoidal shape. The values are lowest in the light period and increase during the dark. Only LA shows more irregularity. As in BT and in HR it exhibits lower values during the light period, but despite the strong smoothing there are several peaks during the night.

Since we were not able to measure absolute values of LA, we had to standardize them by setting the mean value of each animal as 100%. Therefore, the minimal values of LA are always 0%.

Filtration with a cut-off frequency of 40 min

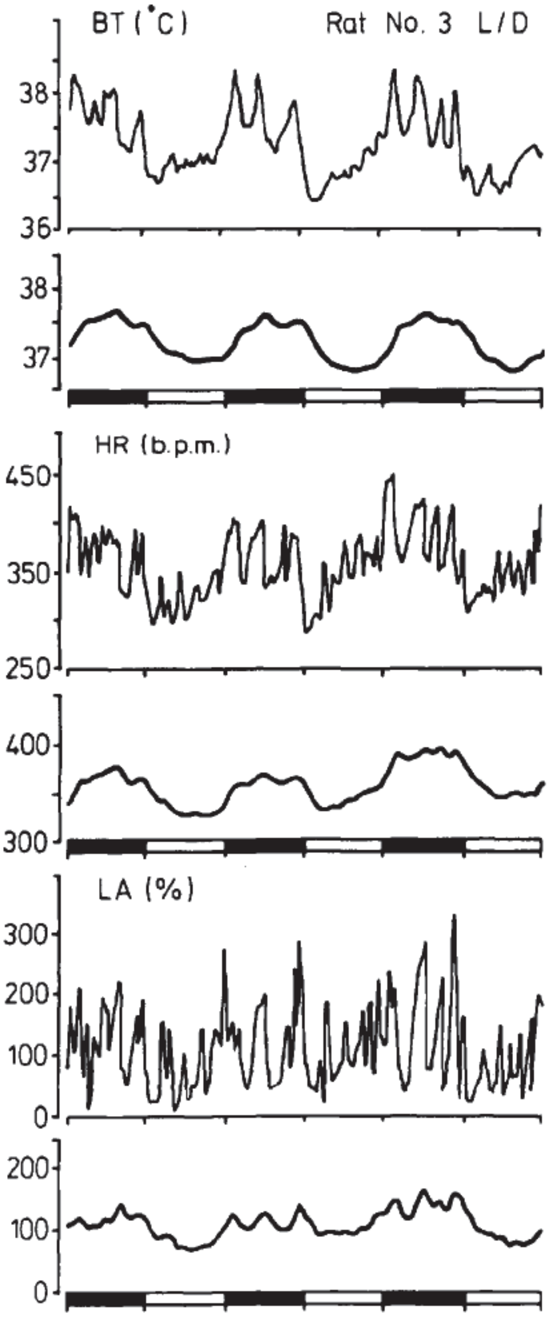

Fig. 1. Fluctuations of BT, HR, and LA simultaneously measured in a single rat (No. 3) over a period of 3 days under a LD cycle of 12:12 hours. 5-min mean values were smoothed by a cut-off frequency of 24 hours (lower line) and a 40 min (upper line). Black sections in the horizontal bar indicate the dark periods (18·00 to 6·00).

reveals a second component of fluctuations which occur simultaneously within the three variables.

LA increases during the dark phase and exhibits several periods of activity and rest. BT and HR also show rapid variations of 3–5 h period length mainly during the dark period.

Daily patterns

Figs 2, 3, 4 illustrate the daily patterns of HR, BT, and LA separately for 5 rats; 30-min averages were employed as units of the graph, standard deviations (SD) between the days plotted as vertical lines. They were computed by averaging

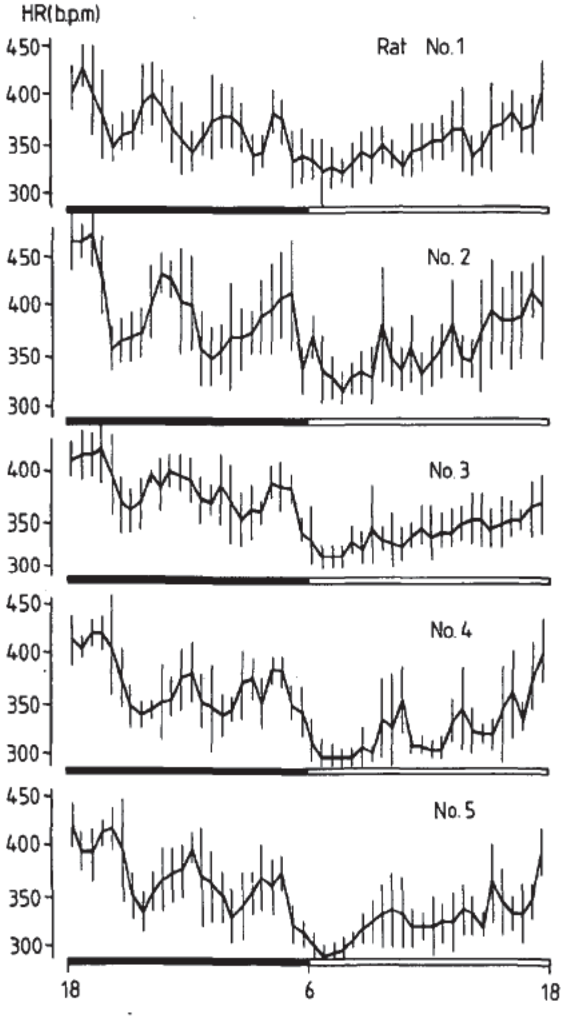

Fig. 2. Daily patterns of HR separately for 5 rats from continuous measurements over 5 days. 30-min averages are employed as units, standard deviations between the days plotted as vertical lines. The black portion in the horizontal bar indicates the dark period.

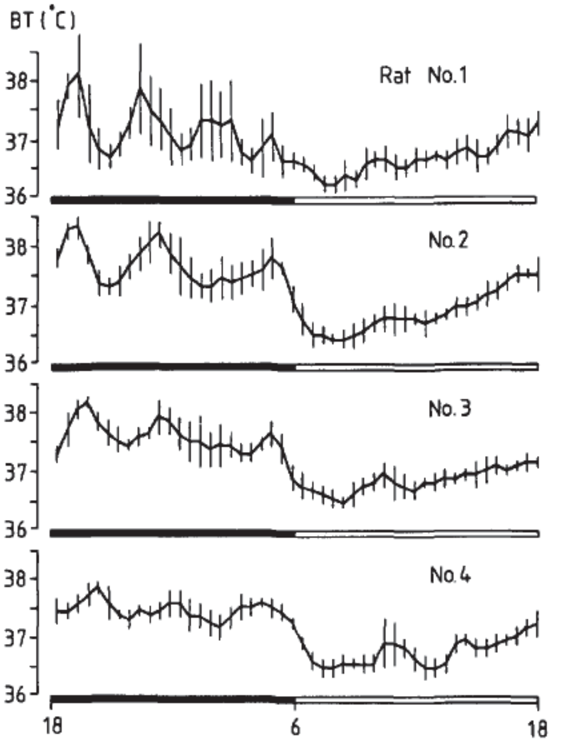

Fig. 3. Daily patterns of BT separately for 4 rats from continuous measurements over 5 days. 30-min averages are employed as units, standard deviations between the days plotted as vertical lines. The black portion in the horizontal bar indicates the dark period.

the 5-min values within each 30-min interval, then calculating a mean value and SD for the coincident 30-min intervals of 5 days. A failure during processing caused the loss of the data of BT for rat No. 5.

HR, BT, and LA show similar daily patterns within the animals. Usually, there were 3 (rarely 4, as shown in animal No. 1) reproducible peaks during the dark period and often another peak 3–4 h after the onset of light. These peaks were caused by the rapid variations of the three variables which occur simultaneously with a period length of 3–5 h.

Obviously there are significant differences between the 30-min values, mainly during the night. But statistical comparison of the 30-min means does not seem to be an appropriate method for proving the regularity of the peaks. This will be done by time series analysis in another study.

The lowest values were obtained during the first hours of the beginning of the light period. BT and HR increased slowly towards the onset of dark, whereas the mean values of LA increased just before the beginning of the dark period.

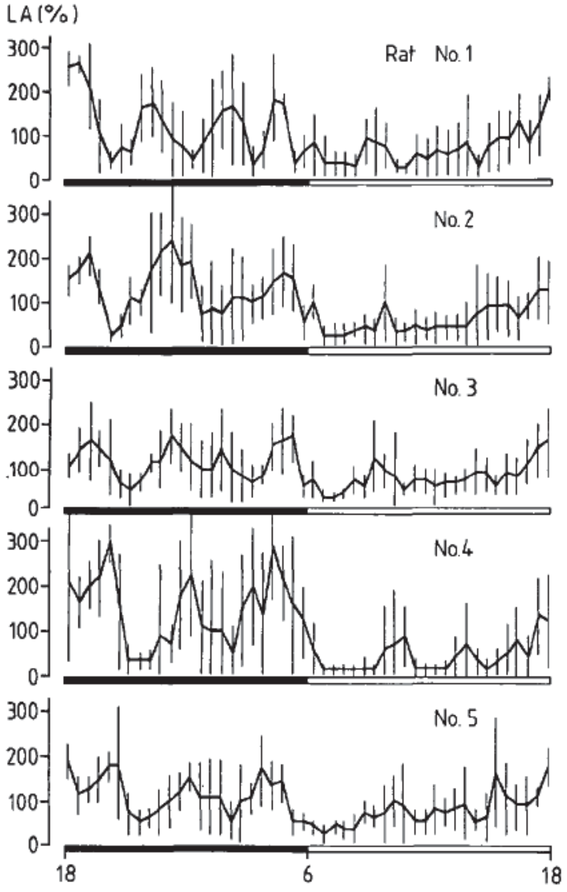

Fig. 4. Daily patterns of LA separately for 5 rats from continuous measurements over 5 days. 30-min averages are employed as units, standard deviations between the days plotted as vertical lines. The black portion in the horizontal bar indicates the dark period.

Because of averaging, minimal and maximal values derived from Figs 2, 3, 4 underestimate the real variability of HR, BT, and LA during the day. This is demonstrated in Fig. 5 which shows means of the minimum, maximum, and mean values of LA, BT, and HR for the dark (hatched bars) and the light period (unhatched bars). They were calculated on the basis of 5-min values by determining the lowest 5-min value (min), the highest 5-min value (max) and 12-h mean values (x) separately within the dark and the light period of each animal and each day. These values were averaged over 5 animals and 5 days. The difference between the minimal and the maximal values represents the band-width of the 5-min values. SD represents the variability between days and animals.

HR shows variations from 286 ± 12 to 470 ± 26 b.p.m. within 24 h. Although the lowest 5-min values of HR are found in the light period and the highest 5-min values in the dark period, there is no obvious difference between the minimal values or the maximal values reached in the dark period and those reached in the light period. The difference between the minimal and maximal values within the light period are nearly in the same range as the differences within the dark period. This means that the band-width of the 5-min values within these two periods is much greater than the differences between day and night. Day–night differences are only visible between the 12-h mean values.

BT is quite similar to HR. The 5-min values of BT varied within 24 h from 36·15 ± 0·15°C to 38·45 ± 0·25°C. BT shows a nearly equivalent variability of the 5 min values within the dark and the light period, but in opposition to HR dark–light differences are also visible for the minimal and maximal values. LA is different. Maximal values occurred in a range of 400–550% mean activity. As described above, the values of LA were standardized by setting the mean value of each animal as 100%. Therefore, the SD demonstrates only the variability between days. The minimal values of LA are always 0%.

Although there are obvious differences of the mean and the maximal values between the light and the dark period, the great variability of LA is based mainly on short-term fluctuations of the amplitude within each period.

Coefficients of correlation

The daily patterns of HR, BT, and LA showed a close correlation to each other. Coefficients of correlation depend highly on the method of calculation. Therefore correlations of LA vs HR, LA vs BT, and HR vs BT were calculated without any modification of the values as well as with a previous smoothing of the variables and with a time shift of the variables towards each other. The correlations of BT vs HR and LA showed an increase of about 0·1 when there was a backward time shift of BT of 10 min relative to the other variables. The correlation of LA vs HR showed no increase due to time shifting.

Smoothing or transformation of the variables can also yield a small increase of correlations. This is not shown here in detail in order to reduce data to a reasonable amount.

A single computation of correlation coefficients on the basis of 5-min values without smoothing or time shifting is shown in Table 1 (column I) separately for each animal and each pair of variables.

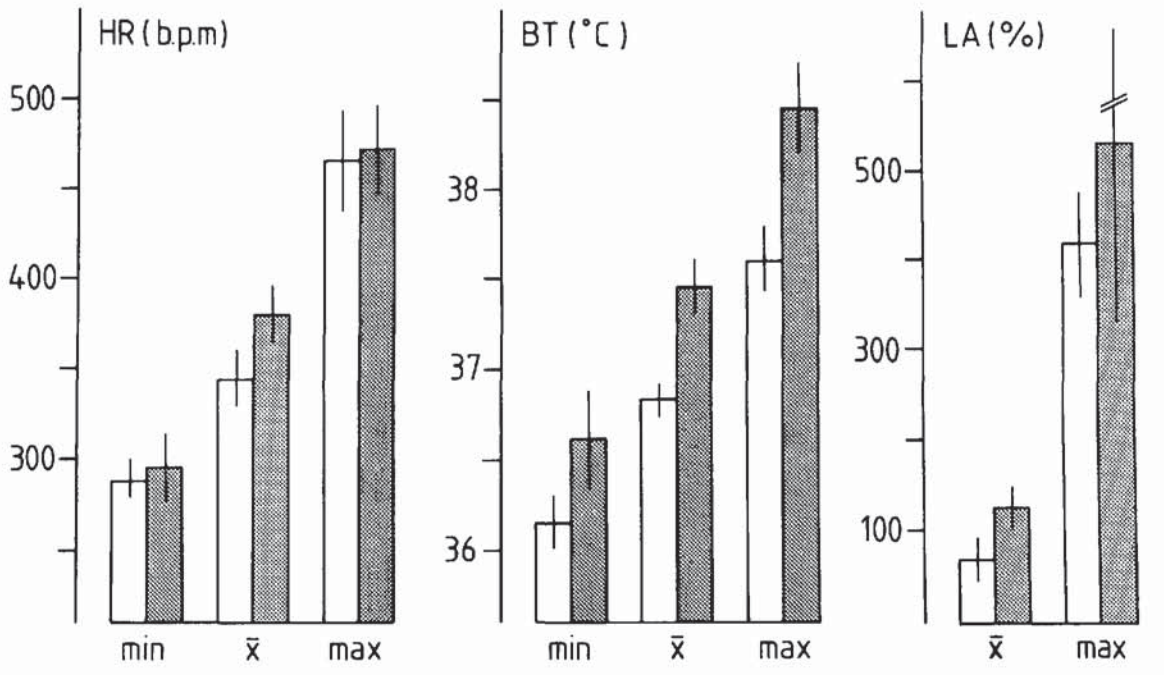

Fig. 5. Minimum, mean and maximum values of HR, BT, and LA determined on the basis of 5-min values. The vertical bars represent mean values of 5 animals and 5 days calculated separately within the dark (hatched bars) and within the light period (unhatched bars). Standard deviation includes variability between days and animals.

In order to distinguish between correlations of short-term fluctuations and correlations of long-term fluctuations we calculated within-hour correlations and between-hour correlations among HR, BT, and LA for each animal. Hourly averages of HR, BT, and LA were employed as units of the between-hour correlations. These correlations are shown in column II. The within-hour correlations were based on 5-min mean values. Because of the improvement of correlation by means of a time shift, values of HR and LA were paired with those of BT 10 min later.

In all cases there are high correlations ($P<0{\cdot}001$) between all variables. For the 5-min values the highest correlations were found for HR vs LA, the lowest ones for BT vs LA.

Between-hour correlations computed on the basis of hourly mean values are higher than the common correlations of 5-min values in all cases. Now HR vs BT correlate as well as HR vs LA.

The within-hour correlations between HR and LA were, with only one exception, nearly as good as the between-hour correlation, whereas within-hour correlations for LA vs BT and HR vs BT are only in a range of 0·27 and 0·35, but also significant. This shows a close interrelationship of the three variables in the rat for short-term as well as long-term fluctuations.

Discussion

Telemetric methods made it possible to study HR and BT continuously over a period of 5 days

Table 1. Coefficients of correlation calculated on the basis of 5-min values (column I) and correlations between hours (column II) and within hours (column III).

Level of significance ($P<0{\cdot}001$): >0·11 (column I), >0·3 (column II), >0·11 (column III).

Animal no.	*LA vs HR*			*LA vs BT*			*HR vs BT*		
	I	II	III	I	II	III	I	II	III
1	0·71	0·77	0·70	0·51	0·79	0·29	0·66	0·83	0·41
2	0·71	0·72	0·70	0·53	0·63	0·32	0·61	0·76	0·34
3	0·69	0·76	0·70	0·40	0·63	0·23	0·65	0·80	0·29
4	0·61	0·81	0·45	0·50	0·75	0·25	0·68	0·83	0·37
5	0·73	0·83	0·70						

without any disturbance of the animals, and their relationships to simultaneously measured LA. As illustrated in Fig. 1, there are two main components of variation in HR, BT, and LA: the 24-h rhythm and fluctuations occurring in intervals of 3–5 h. Most of the variability of the three variables within 24 h depends on these short-term fluctuations (Fig. 5). Differences of minimal, maximal, and mean values between the dark and the light period are rather small.

Simultaneous measurements of BT by a telemetric system and by hand have shown that the handling during a rectal measurement will alter the body temperature of the animal and will yield smaller variations within a day (Miles, 1962) and so telemetric systems are considered to be the most suitable approach for long-term measurements in unrestrained animals. Previous measurements of BT with telemetry equipment in several laboratories (Miles, 1962; Bøjsen, Møller & Faber 1971; Philippens, 1976; Honma & Hiroshige, 1978*a*,*b*,*c*) (36·4°C–38·3°C) agree well with our results (36·3°C–38·5°C).

The measurement of HR entails similar problems as the measurement of BT. The advantage of telemetrical methods in HR measurements in the rat was first demonstrated by Bohus (1974).

Since measurements of LA suffer by not representing absolute values of activity, there are difficulties in comparing our results with others. Nevertheless there are studies which have shown similar short-term fluctuations of LA within a range of 3–6 h in the rat under a LD cycle of 12:12 h (Philippens, 1976; Lemmer *et al.*, 1980) or continuous light (Honma & Hiroshige, 1978*a*,*b*,*c*). The LA pattern found by us for the LEW/Ztm was almost identical to that found by Lemmer *et al.*, 1981) for the same strain.

In our study, the daily pattern of LA is characterized by 3 or 4 reproducible activity peaks during the dark phase and another peak 3–4 h after the onset of light. But these short-term fluctuations, also shown by the TNO strain (Lemmer *et al.*, 1981) and a Wistar strain (Honma & Hiroshige, 1978*a*,*b*,*c*) may not be so obvious in other strains. It is possible that these period lengths are strain-dependent, which prevents us from generalizing our results to 'the rat'. Furthermore, recordings of grouped animals or averaging over several animals may mask individual short-term fluctuations. The daily patterns of HR and BT also showed variations similar to those of LA. Peaks of BT and HR were precisely correlated in their temporal sequence with those of LA.

Significant correlations exist between these three variables (Table 1), even on the basis of 5-min values without time-delay and without averaging or filtering. The between-hour correlation gave similar coefficients as found by Meinrath & D'Amato (1979). But within-hour correlations in the present study are significant, in contrast to those reported by Meinrath & D'Amato (1979).

We agree with Meinrath & D'Amato that short-term changes in HR represent mainly changes in muscular activity. But we do not support the theory that BT and LA follow independent oscillators, which is discussed by Aschoff (1970) and Meinrath & D'Amato (1979). Since calculations with a time shift of BT of only 10 min relative to HR and LA increases the correlations by considerable amounts, the lower coefficients of the within-hour correlations seem to result from a physiological delay of BT rather than an uncoupling of BT from LA and HR.

The increment of BT which reaches the maximum 15 min after the onset of LA (Honma & Hiroshige, 1978*b*) or feeding (Abrams & Hammel, 1965) is in the range of our results.

Nevertheless, the decrease of internal temperature from the liver to the lower abdomen (Grayson & Mendel, 1956), organ-specific heat production during a certain state of activity, and a time delay of the telemetry system should be considered as additional sources of variability in BT measured under these conditions. One must also take into account that the calculations of correlations are done under the assumption of a linearity for the variables.

Furthermore, it has been shown that the correlation of LA with BT follows a circadian rhythm and that the correlation coefficient reaches a maximum in the dark period (Honma & Hiroshige, 1978*b*). Likewise it is possible that BT, and to a lesser degree HR as well, are subject to a physiological smoothing for much larger time intervals than a few minutes. But this is difficult to prove by statistical methods such as time lagging cross-correlations because these long-term trends interfere with the short-term fluctuations. Focussing the problem on data sampling and processing procedures, one could say that the results depend highly on the time intervals and the duration of each data sample. A nearly continuous data sampling at short intervals is necessary because of the rapid alterations of HR and LA in the rat. Sampling at intervals of a few minutes or more for only a few seconds will not be representative. If necessary data can be reduced afterwards by averaging the original values. On the other hand, averaging over long periods may also result in a loss of information. In this case averaging over some hours gives only a sinusoidal shape of the daily pattern.

Some other biological functions, such as hormone secretions, may vary in hourly intervals (Tannenbaum & Martin, 1976; Tannenbaum *et al.*, 1979) and could be timed to bursts of motor activity as Honma and Hiroshige (1978*a,c*) demonstrated in corticosterone secretion. These 'ultradian rhythms' may be an essential part of the daily fluctuations of biological variables.

This poses the question of the appropriate timing of experiments. Although the origin of these fluctuations is still unknown, they may be an important component of variability in results, obtained even under standardized conditions.

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Spontane kurzfristige Änderungen im tageszeitlichen Verlauf von Herzfrequenz, Körpertemperatur und Bewegungsaktivität der Laborratte

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Zusammenfassung
An frei beweglichen Ratten wurden Herzfrequenz (HF), Körpertemperatur (KT) und lokomotorische Aktivität (LA) kontinuierlich über einen Zeitraum von 5 Tagen registriert.

Alle drei Variablen zeigen neben den bekannten circadianen Schwankungen beträchtliche kurzfristige Amplitudenänderungen, die hauptsächlich während der Nacht, aber auch am Tage auftreten. Die Werte der HF variieren im Bereich von 286 ± 12–470 ± 26 Schl./min, die der KT zwischen 36·15 ± 0·15–38·45 ± 0·25°C. Diese große Variabilität von HF, KT und LA im Tagesverlauf beruht nicht so sehr auf Unterschieden zwischen der Licht- und Dunkelphase, sondern vielmehr auf kurzfristigen Änderungen von 3–5 Stunden Periodenlänge.

Alle drei Variablen zeigen einen ähnlichen tageszeitlichen Verlauf innerhalb der Tiere. Im Tagesprofil treten zumeist 3, manchmal auch 4 reproduzierbare Peaks während der Dunkelperiode und ein weiterer Peak 3–4 Stunden nach Einschalten des Lichtes auf. Die auf der Basis von 5 min Mittelwerten errechneten Korrelationskoeffizienten sind hochsignifikant ($P<0·001$) für LA–HF (0·61–0·73), LA–KT (0·40–0·53) und HF–KT (0·61–0·68).

Die Korrelationen zwischen den Stunden sind im allgemeinen noch besser. HF–KT (0·76–0·83) und LA–KT (0·63–0·79) korrelieren nun ebenso gut wie LA–HF (0·72–0·83). Bei den Korrelationen innerhalb der Stunden ergeben sich für LA–KT (0·23–0·32) und HF–KT (0·29–0·41) niedrigere Korrelationskoeffizienten als für LA–HF (0·40–0·70). Dies scheint auf einer physiologischen Verzögerung der KT gegenüber HF und LA zu beruhen. (G)