Simultaneous Determination of Circadian Rhythms of Locomotor Activity and Body Temperature in the Rat

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Abstract Simultaneous determination of the circadian rhythms of locomotor activity and body temperature was carried out in the rat. Deep body temperature was monitored continuously using a telemetric device. The circadian rhythm of locomotor activity was characterized by clustering of several bursts of activity during the dark period. The circadian rhythm of body temperature was also characterized by bursts of small fluctuations which were well correlated with those of locomotor activity. Correlation between the two functions was such that the regression line expressing body temperature as a function of locomotor activity had approximately the same slope for dark and light periods, but a body temperature for a given amount of locomotor activity was significantly higher during the dark period than during the light one. After a prolonged exposure to constant light, the circadian rhythm disappeared in both functions. Both showed bursts of fluctuations which were correlated with each other. These results indicate that the bursts of body temperature increment were dependent on those of the locomotor activity. However, manifestation of the circadian rhythm per se of body temperature could not be explained as resulting exclusively from the circadian fluctuation of locomotor activity.

To study internal relationships among several biological rhythms, it is necessary to determine simultaneously more than one function in the same subject. In studies of chronobiology using small rodents such as rats, only a few papers are available in which several biological rhythms were determined simultaneously (ASCHOFF and VON SAINT PAUL, 1973; SPENCER et al., 1976). The main reason for this seems to be that simultaneous measurements of many functions are possible only with such external restrictions that would disturb the circadian rhythm of experimental animals. To circumvent this difficulty and to obtain information continuously from freely behaving animals, the use of telemetric devices appears to be of vital importance.

The use of a continuously recording device revealed in the rat that the cir-
cadian rhythms of locomotor activity and body temperature were well maintained during the light-dark cycle and under constant dimly illuminated conditions (Halberg, 1965; Richter, 1967). It was also shown in the rat that the bursts of locomotor activity associated with feeding accounted for the increase in body temperature (Abrams and Hammel, 1964) and elevation in oxygen consumption was precisely tied in time to an occurrence of the burst of activity (Morrison, 1968). But since in these studies the body temperature was not telemetered, there is little doubt that the rats responded to the external restrictions. Nevertheless, these studies suggest that there exists a causal relation between locomotor activity and body temperature. On the other hand, studies on human subjects disclosed that dissociation of physical activity from body temperature occurs in a continuously awakened state (Kleitman, 1923), after a sudden phase shift of sleep-wakefulness (Sharp, 1961) or when subjects have been isolated from all environmental time cues (Aschoff et al., 1967).

The purpose of this study is to introduce an efficient telemetric device for monitoring body temperature in combination with a monitoring system for locomotor activity in the same rat and to clarify internal relationships between the circadian rhythms of these two functions.

MATERIALS AND METHODS

Animals and experimental conditions. Male adult rats of Wistar strain, weighing 300–350 g, were used. Animals were bred and reared in a controlled animal room where the environmental conditions were kept constant (temperature, $22 \pm 1^\circ$C; humidity, 60%; light, 06:30–18:30 hr; dark, 18:30–06:30). The animals were transferred to an isolation room at the age of 30–40 days. The environmental conditions of the isolation room were the same as those of the animal room. The rats were housed in individual plastic cages and fed commercial chow and water ad libitum. The size of the cages was $36 \times 30 \times 16$ cm and the light intensity at the surface of the cage was 200 lux. After having been reared in the isolation room for at least two weeks, the animals were subjected to simultaneous measurements of body temperature and locomotor activity.

Measurement of locomotor activity. Spontaneous locomotor activity of the rat was measured by a FARAD Animex type S. The principle of measurement was as follows: A resonance circuit of an electric oscillator was placed beneath the floor of the cage so that an electric signal could be obtained each time a rat moving on the floor of the cage crossed the resonance circuit. The number of times the rat crossed the resonance circuit during a period of 15 min was expressed as counts/15 min and taken as an index of locomotor activity.

Measurement of body temperature. A telemetric system was used for detection of deep body temperature. The system was composed of a thermistor, a transmitter, an FM receiver, a demodulator and a recorder. A thermistor (43B11,
Ohizumi Instr.) was surgically implanted in the retroperitoneal cavity of the rat. The lead wires from it were covered with a polyethylene tube and coated with silicone. They were connected to a transmitter attached to the back of the animal. The transmitter and power source were fixed in place on a small plate with epoxy glue. As the power supply a mercury battery (H–B 1.3 volts, National) was used which was easily replaceable. The transmitter contained a carrier oscillator and a multivibrator circuit which varied the pulse width in response to resistance changes in the thermistor (pulse interval modulation). The size of the transmitter was $35 \times 20 \times 8$ mm and the weight was about 10 g. The transmitted radio waves were detected by an ordinary FM receiver and decoded by a demodulator. An FM antenna was wound round the upper edge of the cage and no directivity was observed within a range of 1 m. Specifications of the system were as follows: thermal time constant, 5 sec; range of measurement, 32–42°C; operating spectrum, 76–85 MHz; transmission distance, 1–2 m. The operation life of the battery was about 3 days. Calibration of the thermistor was performed using a large volume water bath. The resolution power of the thermistor was 0.01 ºC. With the whole system the body temperature was measured with an accuracy of 0.05°C. Although body temperature was continuously monitored, the data presented below were those sampled at 15 min intervals.

**Experimental procedures.** Attachment of the telemetry device was performed aseptically in 5 rats (Nos. 1–5) under pentobarbital anesthesia. To prevent infection, chlortetracycline powder, dissolved in drinking water, was administered for 4 days after surgery. After one week of postoperative recovery, locomotor activity and body temperature were recorded continuously for 3–7 days. Two other rats (Nos. 6 and 7) were exposed to 200 lux of continuous illumination for 3 months. Then operation for attachment of the telemetric device was conducted and after a lapse of 7 days continuous measurements of locomotor activity and body temperature were initiated.

**Statistical analysis.** Two-tailed F-test was used for comparison of regression lines when necessary.

**RESULTS**

*Circadian rhythms of locomotor activity and body temperature in a single rat in light-dark cycles*

An example of simultaneous determination of circadian rhythms of locomotor activity and body temperature is shown in Fig. 1. Activity bursts of 1–2 hr duration were observed during the dark periods. Apparently, a clustering of the bursts at night is the main feature of the circadian rhythm of locomotor activity. Similarly, body temperature showed two components of fluctuation: one, small rapid undulations which seemed to be tied in time with the burst of locomotor activity, and the other, slow undulations which corresponded with the light-dark cycle.
Fig. 1. Simultaneous determination over a 48-hr period of locomotor activity and body temperature in a rat during two cycles of light and dark. Black portions in the horizontal bar indicate the dark period.

The range of variation in body temperature was about 1°C.

The correlation between locomotor activity and body temperature

Using data accumulated for more than two cycles of light-dark alteration in a total of 5 rats, we investigated the statistical correlations between body temperature and locomotor activity. It was found that when the counts of locomotor activity were plotted against body temperature values obtained 15 min after the former, a significant correlation was seen between the two functions. In Fig. 2 are presented all data obtained from the 5 rats in a form of a correlation diagram. The body temperatures during the dark period and those during the light period are plotted with filled and open circles, respectively. Regression lines are also drawn for light and dark separately. It is shown that in each rat the regression line for the dark period is displaced upwards relative to that for the light period, but the two lines have almost the same slope (regression coefficient). Table 1 indicates the values of slopes and levels of each regression line (the term “level of regression line” indicates the ordinate value of the line for zero of the abscissal axis). The two-tailed $F$-test disclosed a significant difference in the levels of the regression lines between the dark and light periods, but no difference was found in the slope. The data from rat No. 5 in which no significant correlation of the two functions was proved for the light period were not used for the $F$-test.

Although Table 1 shows that in each rat the correlation between locomotor activity and body temperature was significantly high during the dark period, it was found that such correlation was not kept constant throughout the dark period. This was shown in the following way. From each series of measurements of body temperature ($T$) and locomotor activity ($A$), we calculated the differences between successive measurements at 15 min intervals ($\Delta T$ and $\Delta A$). Each series

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Table 1. Correlation between locomotor activity and body temperature during the light (L)-dark (D) cycle.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Illuminating conditions</th>
<th>No. of determinations</th>
<th>Slope ($\times 10^{-3}$)</th>
<th>Level</th>
<th>r</th>
<th>Level</th>
<th>r</th>
<th>Slope</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>48</td>
<td>0.82</td>
<td>36.68</td>
<td>0.460</td>
<td>p&lt;0.005</td>
<td>N.S.</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>47</td>
<td>1.01</td>
<td>37.30</td>
<td>0.661</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>L</td>
<td>48</td>
<td>0.99</td>
<td>36.61</td>
<td>0.410</td>
<td>p&lt;0.005</td>
<td>N.S.</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>47</td>
<td>0.87</td>
<td>37.21</td>
<td>0.719</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L</td>
<td>48</td>
<td>0.86</td>
<td>36.36</td>
<td>0.336</td>
<td>p&lt;0.025</td>
<td>N.S.</td>
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<tr>
<td></td>
<td>D</td>
<td>47</td>
<td>1.07</td>
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<td>0.802</td>
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<td></td>
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<td>36.44</td>
<td>0.519</td>
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<td>N.S.</td>
<td>p&lt;0.005</td>
<td></td>
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<tr>
<td></td>
<td>D</td>
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<td>37.02</td>
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<td>p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
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<td>0.267</td>
<td>p&lt;0.100</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td></td>
<td>D</td>
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<td>37.33</td>
<td>0.575</td>
<td>p&lt;0.001</td>
<td>(N.S.)</td>
<td>—</td>
<td>—</td>
</tr>
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</table>

* Rats were the same as in Fig. 2. Slope, regression coefficient. Level, estimated value of body temperature for zero of locomotor activity. r, correlation coefficient between locomotor activity and body temperature. In the right three columns are given probabilities to estimate statistical significance for r (t-test) and for differences of slopes and levels between light and dark (two-tailed F-test).

Fig. 2. Correlation diagrams between locomotor activity and body temperature with regression lines. Body temperature paired with a given locomotor activity is the one obtained 15 min after the latter. Ordinates, body temperature (°C). Abscissae, locomotor activity (counts/15 min). Solid circles, dark period. Open circles, light period.
of $\Delta T$ and $\Delta A$ thus obtained was divided into segments of 1 hr period. The data from all the rats for the same segment of the dark period were pooled and subjected to calculation of the correlation coefficients between $\Delta T$ and $\Delta A$. Figure 3 shows changes of the $\Delta T-\Delta A$-correlation-coefficient for the entire period of dark, including 1 hr period each before and after it. It is seen that the $\Delta T-\Delta A$-correlation-coefficient increases after the beginning of the dark period, reached the maximum around the middle stage (00:30–01:30), and reduced to an insignificant level in the last stage (05:30–06:30).

**Fluctuations of locomotor activity and body temperature in rats exposed to constant light for 3 months**

Figure 4 illustrates changes of locomotor activity and body temperature in a rat exposed to 200 lux continuous light for 3 months. Apparently, the circadian rhythm was obscured in both body temperature and locomotor activity. Only bursts of locomotor activity of about 2 hr duration appeared with a regular interval of 3–4 hr. It is noteworthy that body temperature also changed in accordance with the changes of locomotor activity. The changes of body temperature were significantly ($p<0.001$) correlated with those of locomotor activity, as shown in Fig. 5.

**DISCUSSION**

The telemetric system used for continuous measurement of body temperature...
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Fig. 4. Fluctuations of locomotor activity and body temperature in a rat exposed to continuous illumination for 3 months. Times given below the horizontal bar indicate the local standard time.

Fig. 5. Relationship between locomotor activity and body temperature in rats exposed to continuous light for 3 months. Body temperatures plotted are those obtained 15 min after the corresponding locomotor activities. Regression lines and regression coefficients \( r \) are given in the figures.

in this study was in principle based on pulse interval modulation. The advantage of this circuit lies in its being insensitive to small changes in the source voltage. Therefore, neither drift nor damping of the baseline occurred during a prolonged course of measurement. Indeed, a drift of the baseline was kept less than 0.05°C
for more than 24 hr. Although the operation life of the battery is relatively short, it could easily be replaced. The resolution power of the system and the temperature range registered were adequate for the present study of the circadian rhythm of body temperature. The thermistor and lead wires implanted in the abdominal cavity were sufficiently small, so that digestive or kidney functions were not disturbed. The experimental animals gained weight and showed a pattern of locomotor activity similar to that seen in non-operated animals. At autopsy, the thermistor and lead wires were found to be surrounded by a thin transparent fibrous layer, suggesting that the animal's reaction to the implanted materials were non-specific.

Continuous and simultaneous recordings of several functions are necessary for examining causal relations or internal phase angles of the circadian rhythms. Telemetry at present is considered to be the most suitable approach for this purpose. The circadian rhythm of body temperature in the rat has been investigated by telemetry in several laboratories (e.g., Halberg, 1965; Bjoøsen et al., 1971) and the results were compared with other functions determined simultaneously (Spencer et al., 1976). Abrams and Hammel (1964) reported that feeding was consistently associated with a rapid increase in the temperature of the brain and abdominal cavity in the rat. Morrison (1968) demonstrated that all elevations in oxygen consumption above the baseline were precisely related in time to the occurrence of bursts of activity. However, these investigators apparently did not give due consideration to the relationship between the circadian rhythms of locomotor activity and body temperature.

As illustrated in Fig. 1, the two main components of fluctuation were distinguished from each other in locomotor activity and body temperature, namely, short-lasting burst and 24-hr rhythm. The regular bursts of locomotor activity with a period of about 2 hr have been reported to occur in most species (Aschoff, 1962), but a detailed analysis has not yet been made with respect to their relation to body temperature changes over a period of 24 hr. The present study demonstrated that there is a significant positive correlation between locomotor activity and body temperature (Table 1). The correlation was not definitely significant when the two variables observed at the same instants were used for calculation. For example, the correlation coefficient (r) in rat No. 1 during the dark period was 0.159 (t = 1.08, N.S.) when calculations were made by pairing the values observed at the same instants, but it was as high as 0.661 when the values of body temperature were paired with those of locomotor activity 15 min preceding the former. Calculation with a more than 30 min delay of body temperature relative to locomotor activity did not yield a significant correlation between the two functions. Similar results were obtained from the other rats (data are not shown). The increment in body temperature has been reported to reach the maximum at 15 min after the onset of locomotor activity of feeding (Abrams and Hammel, 1964). This would be the basis for our finding that locomotor activity is signifi-
cantly correlated with body temperature measured 15 min later than the former. The slopes of regression lines for the dark and light periods were essentially the same, indicating that the manner in which the increase of locomotor activity results in an increase of body temperature is fundamentally the same for the two periods. However, the levels of body temperature during the dark period were significantly higher than those during the light period. This indicates that an increase of body temperature during the dark period can not be explained solely in terms of an increase of locomotor activity. In other words, the circadian rhythm of body temperature is not a direct result of the circadian rhythm of locomotor activity. Heusner (1956) found a similar relation between oxygen consumption and locomotor activity in rats. He demonstrated a nocturnal augmentation of oxygen consumption, not connected with an increase of body movement; oxygen consumption during night was elevated 12–30% over the diurnal level at rest. To this value was added an extra-consumption of oxygen which was directly proportional to the spontaneous activity.

Body temperature did not show a significant correlation with increments of locomotor activity in the last phase of the dark period as illustrated in Fig. 3. This appears to indicate that body temperature did not necessarily rise during this period even when body movements increased. Thus it becomes necessary to postulate a participation of another mechanism for the regulation of the circadian rhythm of body temperature.

A large circadian oscillation of temperature was shown in human subjects kept at rest for the entire experimental period (Aschoff and Pohl, 1970). Sharp (1961) reported a transient dissociation of two circadian rhythms: following a 12-hr shift of routine life, it took 3–4 days for the temperature rhythm to adapt to a new cycle. These results indicate that the temperature variation was not directly dependent on physical activity. Deep body temperature is determined by a balance between heat production and heat loss. Hildebrandt and Engelbertz (1953) reported that the daily variation in skin temperature showed almost a mirror image of the change in rectal temperature. Aschoff and Heise (1972) demonstrated the existence of daily rhythm in heat conduction in resting men and concluded that 75% of circadian variation in core temperature was due to variation of heat loss, the remaining 25% being due to the change in heat production. In our study the circadian rhythm of heat loss was not monitored. Nevertheless, it is likely that in the rat too, a daily change of heat loss mechanism participates in the regulation of the circadian rhythm of body temperature.

The circadian rhythms of locomotor activity and body temperature are known to run freely with slightly different periods from 24 hr in the absence of all Zeitgebers. Aschoff et al. (1967) demonstrated an internal desynchronization between the free-running rhythms of locomotor activity and rectal temperature in men who were isolated from time cues. Aschoff and von Saint Paul (1973) also demonstrated in the chicken that the free-running rhythm of brain temperature persisted.
after the circadian rhythm of locomotor activity had been abolished in constant dim illumination. They postulated that the circadian rhythms of locomotor activity and body temperature were regulated by different oscillators. In the rat exposed to 200 lux continuous light, however, the same internal phase angle difference was preserved between these two functions. This suggests that the two rhythms are coupled to a common oscillator (Honma and Hiroshige, 1978a). Prolonged exposure to continuous illumination decomposed the circadian rhythm of locomotor activity into bursts occurring over a 24-hr period (Honma and Hiroshige, 1978b). As shown in Fig. 4, the circadian rhythm of body temperature also is obscured under prolonged continuous light, resulting in bursts of rapid fluctuation. The analysis of the relationship between the two variables (Fig. 5) revealed that body temperature was devoid of the circadian rhythm under this condition and rapid undulations appeared as a direct consequence of changes in locomotor activity.

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REFERENCES


Jap. J. Physiol.


