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# Ambient temperature dependence of the periodic respiratory pattern during long-term hibernation in the garden dormouse, Eliomys quercinus L.

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The periodic respiratory pattern in hibernating garden dormice was studied at $T_A$s of 0, 6.5 and 9° C during six months in each study winter. The results are compared with those obtained in optimal conditions at 4° C. The animals were kept in constant conditions in darkness without food or water.

The periodic respiratory pattern was $T_A$ dependent. Both apnoeic and respiratory periods shortened from 4 to 9° . Deviating from this trend, at a $T_A$ of 0°, the apnoeas were short and the respiratory periods of long duration. The changes in the periodic respiratory pattern at different $T_A$s were parallel to the changes in the periodicity of hibernation.

The periodic respiratory pattern is the most sensitive indicator of undisturbed hibernation at low $T_A$s: 0–6.5° C, but at 9° heart rate is more sensitive.

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## 1. Introduction

During undisturbed hibernation periodic respiration seems to be as characteristic of mammalian hibernation as are periodic arousals. The list of species in which the periodic respiratory pattern has been verified (cf. Pajunen 1974, 1976) includes the common dormouse, woodchuck, hazel-mouse, bats, Finnish and German hedgehogs, golden hamster, the eastern and western subspecies of the garden dormouse, 13-lined ground squirrel, birch mouse, and according to Panuska (1959) the eastern chipmunk.

The aim of the hibernation study should be to obtain results from undisturbed animals. Unfortunately, the handling of animals during experiments easily disturbs them, at least in some species. Hence, it is important to know on what occasion the animals are disturbed, so that the results from disturbed animals can be discarded. There are two types of disturbances: transient ones, and those leading to an arousal. Special attention should be paid to changes in different parameters during or ensuing from small disturbances, appearing for a known or an unknown reason and to the possibility of detecting the most sensitive indicator of undisturbed hibernation.

Hibernating garden dormice are easily disturbed. At 4° C periodic respiratory pattern is the most reliable indicator of undisturbed hibernation (Pajunen 1970, 1974). In studies at different $T_A$s a $T_A$ of 4° was optimal for long-term hibernation in this animal. Body temperature and the mean duration of the hibernation periods are $T_A$ dependent (Pajunen 1983b). The aim of the present study was to compare the periodic respiratory pattern at different $T_A$s in the garden dormouse and to study this pattern as an indicator of an undisturbed hibernating animal.

## 2. Materials and methods

Garden dormice, captured in France, and their offspring reared in the laboratory in Finland by non-sibling mating were used in the experiments (cf. Pajunen 1974). Hibernation was studied during about six winter months at the following $T_A$s: 0 (±1), 6.5 (±0.5) and 9 (±0.5) °C. The results are compared with those obtained at 4 (±0.5) °C (Pajunen 1974). The $T_A$ dependence of the body temperature ($T_B$) and of the mean duration of the hibernation periods during the same studies is discussed by Pajunen (1983b).

The experimental conditions were identical with the earlier studies (Pajunen 1970, 1974, 1983b). The animals were kept undisturbed in constant conditions, in darkness without food or water.

Periodic respiration was recorded on a kymograph (Pajunen 1970) or identified from heart rate recordings (Pajunen 1974). Kymographic records were made directly with a straw attached to the flank of the animal at the site where the respiratory movements were strongest. Heart rate was recorded continuously with chronical silver wire electrodes. The duration of respiratory and apnoeic periods could be measured from heart rate recordings, as the muscle potential generated in the respiratory movements was also recorded. Recordings were made at the beginning of the hibernation season at 0 and 9° C, in midwinter at all $T_A$s, and towards the end of the season at 9° C. Pajunen (1974) includes data from the corresponding three phases of the hibernation season at 4° C.

In order to verify that the results are from undisturbed animals, special attention has been paid to changes in the respiratory rhythm. Comparisons were also made with continuous subcutaneous anterior $T_B$ measurements and with the duration of the hibernation periods (Pajunen 1974, 1983b), both with heart rate recordings (Pajunen 1974, 1983a) from the same study seasons in order to detect the most sensitive indicator of undisturbed hibernation. In order to be able to recognize transient disturbed phases, records of long duration are required.

## 3. Results

The results from respiratory measurements at different $T_A$s are given in Tables 1-4. The durations of the respiratory and apnoeic periods at the beginning of the hibernation season at 0° C varied erratically and it was impossible to calculate mean values. For this reason, results from the beginning of winter and midwinter are presented in different Tables.

At 0° C, at the beginning of the season, there existed signs that this $T_A$ is near the lower limit for periodic respiration in the garden dormouse. At 0° C, after the start of the respiration recordings, the animals sometimes breathed continuously from 0.5 to 5 h, but no other signs of possible disturbance could be detected. Then, very short apnoeas began to occur between long continuous respiratory periods. These respiratory periods began with slow respiration and continued with faster respiration, but not, however, in a regular manner. Thereafter followed longer apnoeic periods and shorter respiratory periods, in which during the first half or first two-thirds, the animals breathed very slowly (less than one respiration movement/min), as in Fig. 1. These may be features occurring at $T_A$ limits at which the animals go over to periodic respiration during hibernation. The durations of the apnoeic and respiratory periods at the beginning of the hibernation season at 0°, registered during a single hibernation period in each animal, are seen in Table 1. Animal 52 had relatively long respiratory periods. Sporadically animals 46 and 50 also had long respiratory periods of 0.5-17 hours duration. The duration of apnoea in animals 46 and 50 varied over a wide range; animal 52, with basically short apnoeic periods, intermittently went through apnoeas of 10 min duration.

In midwinter at 0° C the respiratory pattern was very clearly periodic, with shorter respiratory periods and a narrower range of apnoea duration (Table 2). The duration of apnoea seemed to increase during a single hibernation period. In both animals recordings were made during a single hibernation period. After the commencement of the first recording the animals breathed continuously for a duration of 21-26 min. Once, animal 51 breathed for seven

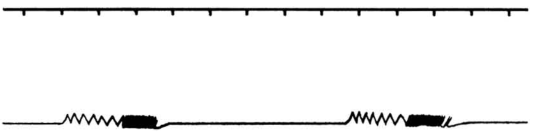

Fig. 1. Kymograph record of periodic respiration in animal No. 46 at the beginning of the hibernation season at 0° C. Time marks at five-minute intervals are shown in the upper line. Below are shown 13- and 14-minute respiratory periods with a 25-minute apnoea between them.

Table 1. Ranges of the duration of the respiratory and apnoeic periods at the beginning of the hibernation season at 0° C.

| Animal | Starting date | Length of record (days) | Respiratory period (min) | Apnoeic period (min) |
|---|---|---|---|---|
| ♂ 52 | 4. Nov. | 3.13 | 11-396 | 1.0-4.5 |
| ♀ 50 | 7. Nov. | 4.05 | 4.0-39 | 1.0-25 |
| ♀ 46 | 12. Nov. | 0.09 | 4.5-6.5 | 1.0-4.0 |
| | 13. Nov. | 3.97 | 3.0-15 | 0.5-53 |

Table 2. The mean duration of the respiratory and apnoeic periods with standard errors of the means and standard deviations in midwinter at 0° C.

| Animal | Starting date | Length of record (days) | Respiratory period (min) Mean | *SE* | *SD* | Apnoeic period (min) Mean | *SE* | *SD* |
|---|---|---|---|---|---|---|---|---|
| ♂ 51 | 12. Feb. | 0.25 | 3.5 | 0.26 | 1.3 | 11.2 | 1.49 | 7.0 |
| | 13. Feb. | 1.06 | 2.8 | 0.06 | 0.5 | 14.1 | 0.52 | 4.9 |
| | 16. Feb. | 2.28 | 3.3 | 0.05 | 0.6 | 20.9 | 0.59 | 6.8 |
| ♀ 46 | 14. Feb. | 0.21 | 3.8 | 0.26 | 1.9 | 1.9 | 0.14 | 1.0 |
| | 14. Feb. | 0.72 | 3.8 | 0.10 | 0.9 | 8.6 | 0.44 | 3.8 |

Table 3. The mean duration of the respiratory and apnoeic periods with standard errors of the means and standard deviations in midwinter at 6.5° C.

| Animal | Starting date | Length of record (days) | Respiratory period (min) Mean | *SE* | *SD* | Apnoeic period (min) Mean | *SE* | *SD* |
|---|---|---|---|---|---|---|---|---|
| ♂ 94 | 17. Feb. | 1.14 | 1.42 | 0.06 | 0.22 | 24.9 | 1.7 | 10.8 |
| ♂ 57 | 25. Feb. | 1.06 | 0.90 | 0.02 | 0.20 | 22.9 | 0.6 | 4.9 |
| ♂ 72 | 18. Feb. | 1.11 | 1.28 | 0.04 | 0.23 | 30.0 | 2.0 | 11.0 |

Table 4. The mean duration of the respiratory and apnoeic periods with standard errors of the means and standard deviations at 9° C. The data are grouped into three study seasons.

| Animal | Starting date | Length of record (days) | Respiratory period (min) Mean | *SE* | *SD* | Apnoeic period (min) Mean | *SE* | *SD* |
|---|---|---|---|---|---|---|---|---|
| ♂ 52 | 30. Nov. | 1.00 | 0.33 | 0.01 | 0.11 | 8.4 | 0.18 | 2.3 |
| ♀ 29 | 9. Dec. | 0.93 | 0.64 | 0.03 | 0.28 | 14.6 | 0.71 | 6.6 |
| ♀ 29 | 28. Jan. | 1.14 | 0.84 | 0.03 | 0.25 | 19.7 | 0.45 | 4.0 |
| ♂ 52 | 29. Jan. | 0.90 | 0.88 | 0.02 | 0.16 | 24.5 | 0.96 | 6.9 |
| ♂ 34 | 30. Jan. | 1.06 | 0.64 | 0.01 | 0.11 | 20.0 | 0.23 | 2.0 |
| ♀ 29 | 22. March | 1.03 | 0.31 | 0.01 | 0.17 | 8.4 | 0.27 | 3.5 |
| ♂ 52 | 30. March | 0.74 | 0.51 | 0.02 | 0.19 | 9.8 | 0.40 | 4.1 |
| ♂ 34 | 31. March | 0.92 | 0.30 | 0.02 | 0.18 | 9.2 | 0.37 | 4.4 |

hours without any distinct apnoea, while in animal 46 continuous respiratory periods of 0.5 h duration were recorded twice. These long respiratory periods were excluded from the calculation of mean values. At other $T_A$s, continuous respiratory periods of this duration were never recorded during deep hibernation.

In midwinter at 6.5° C the mean duration of apnoea was longer than at 0°, and that of the respiratory periods shorter (Table 3). In midwinter at 9° C, the mean duration of both apnoeic and respiratory periods was distinctly shorter than at 6.5 ° C (Table 4). The midwinter mean durations of the apnoeic and respiratory periods at $T_A$s of 0, 6.5 and 9° C were tested by analysis of variance and the differences were significant at the 1 % and 0.1 % levels. There was seasonal variation in the mean duration of the apnoeic and respiratory periods at 9° C, the maximum mean duration being in midwinter (Table 4). Tested by analysis of variance, the differences were significant at the 1 % and 5 % levels.

The garden dormouse is relatively easily disturbed during deep hibernation. There were both seasonal differences and differences at

Table 5. The mean duration of the respiratory and apnoeic periods in different animals at different $T_A$s in midwinter, including the data from 4° C (Pajunen 1974).

| $T_A$ °C | Respiratory period (min) | Apnoeic period (min) |
|---|---|---|
| 0 | 2.8-3.8 | 1.9-20.9 |
| 4 | 1.3-3.3 | 36.1-90.3 |
| 6.5 | 0.90-1.4 | 22.9-30.0 |
| 9 | 0.64-0.88 | 19.7-24.5 |

different $T_A$s in the susceptibility to small mechanical disturbances. The animals were most susceptible during a transition period (Pajunen 1974) at the beginning of the hibernation season. Especially at 0° C the animals were difficult to handle at this time. At a higher $T_A$: 9° C the animals were as susceptible in midwinter as during the transition period. However, towards the end of the season they could be exceptionally insensitive to mechanical distrubances. An arousal process was initiated only when the animal was turned on to the other flank.

Of the parameters studied ($T_B$, respiratory pattern, heart rate), the periodic respiratory pattern is the most reliable indicator of undisturbed hibernation at 0, 4 and 6.5° C. Changes in the pattern were always the first sign that the animal was disturbed. Small disturbances caused only a transitory break in the apnoeic period.

At a higher $T_A$: 9° C the animals reacted differently. Simultaneous respiratory and heart rate recordings revealed that heart rate was more sensitive to change after slight mechanical disturbances than respiratory pattern. Heart rate sometimes transitorily rose from under 20 times/min to 100 times/min. Changes in the periodic respiratory pattern were not necessarily present, and arousal was not induced. Only if there existed a shortening of several apnoeic periods and clearly deeper respiration movements during respiration periods, did arousal result. At lower $T_A$s, elevated heart rates (100 times/min) occurred only in connection with a continuous respiratory pattern and led to an arousal. Only during a transition period at 4° C, a small mechanical disturbance sometimes induced a slight heart rate acceleration with the usual response, a transitory break in the apnoeic period (Pajunen 1974). However, during this time high heart rates (23 times/min compared with the usual 7-13 times/min) occurred in some animals without any disturbance factor being discernible.

## 4. Discussion

The periodic respiratory pattern is maintained during undisturbed hibernation in the garden dormouse at $T_A$s of 0–9° C. During entrance into a single hibernation period at 4° C, periodic respiration begins when the $T_B$ sinks to about 20° (Pajunen 1970). At the end of the hibernation period the change to continuous respiration occurs before the $T_B$ begins to rise (Pajunen 1970). This change is relatively sharp in the French animals (Pajunen 1974), but in the Finnish animals apnoeas can become considerably shortened even 24 hours before arousal (Pajunen 1970).

The results from 4° C (Pajunen 1974) and the present results indicate that the periodic respiratory pattern is the most sensitive indicator of undisturbed hibernation at low $T_A$s: from 0 to 6.5° C. At a $T_A$ of 9° C, on the other hand, heart rate was more sensitive to change after small mechanical disturbances than was periodic respiration. This is presumably a consequence of the relatively higher sensitivity of the sympathetic nervous system at higher temperatures. In the study of the periodicity of hibernation, a $T_A$ of 9° C was observed to be near the higher $T_A$ limit for long-term hibernation in the garden dormouse (Pajunen 1983b).

In the periodic respiratory pattern seasonal differences existed at 0 and 9° C. At 6.5° C a midwinter pattern only was recorded. At 4° C in French garden dormice the duration of the apnoeas varied individually and erratically, but the respiratory periods were found to be significantly longer at the beginning of the hibernation season (Pajunen 1974). At 4° C in Finnish animals the apnoeas were significantly shorter during the transition period at the beginning of the hibernation season and were longest and most regular in midwinter, when the hibernation periods were at their longest. During a single hibernation period the apnoeas were longest in the middle of the period. Long apnoeas occurred together with long respiratory periods, and vice versa (Pajunen 1970). A detailed comparison of the breathing pattern in the two subspecies of the garden dormouse is given in Pajunen (1974).

The changes in the periodicity of hibernation were parallel with changes in the respiratory pattern at different $T_A$s during the same study winters. The duration of the hibernation periods was clearly $T_A$ dependent (Pajunen 1983b). A $T_A$ of 4° C was optimal for hibernation in the garden dormouse (Pajunen 1983b). The periodic respiratory pattern was also clearly $T_A$ dependent, and at a $T_A$ of 4° C apnoeas were of maximal length. The mean duration of the respiratory and apnoeic periods in different animals at different $T_A$s in midwinter, including a $T_A$ of 4° C (Pajunen 1974), as presented in Table 5, was tested by analysis of variance and the differences were found to be highly significant. The mean duration of the hibernation periods (Pajunen 1983b), and both the mean duration of the apnoeic and respiratory periods, shortened from 4 to 9° C. The $T_A$ of 0° C deviated from this trend. The hibernation periods were shorter than at 4 and 6.5° C, and this $T_A$ was near the lower long-term limit for hibernation in the garden dormouse (Pajunen 1983b). The apnoeas were at their shortest, and the respiratory periods at their longest, compared with those registered at other $T_A$s. At the beginning of the hibernation season there existed signs that a slight lowering of the $T_A$ would cause a change in the periodic respiration to a continuous type. In preliminary experiments at -2° C the animals seemed to breathe continuously during that time.

In Pajunen (1983b) the difference between short- and long-term studies was emphasized. When the duration of the hibernation periods and the respiratory pattern in short- and long-term experiments at 0° C is compared, differences in the same direction are detected (Pajunen 1979, 1983b, present study). During a short experiment at 0° C, after transference from 4°, the mean duration of the hibernation, apnoeic and respiratory periods was longer. In this short experiment great individual variation was detected in the duration of the apnoeic periods, as the apnoeas lasted in different animals 7.0–74.1 min, in contrast to 1.9–20.9 min in the long-term study. The respiratory period lasted 4.9–7.7 min during the short experiment and 2.8–3.8 min in the long-term study. From the results obtained during the short experiment it can also be concluded that small changes in the $T_A$ do not have such a marked effect on the periodic respiratory pattern as mechanical disturbances.

In other hibernating species periodic respiration has been studied in detail in the hedgehog, *Erinaceus europaeus* and in the golden hamster, *Mesocricetus auratus.* In the hedgehog (in midwinter, when the hibernation periods are longest) apnoea lasted at 4° C about 1.0 to 1.3 hours, with breathing periods lasting about 4 min (Kristoffersson & Soivio 1964b, Tähti 1975). Short apnoeas (10 min) were recorded at 10° C after transference from 4°, but this was in induced hypothermia commenced during summer (Kristoffersson & Soivio 1964c). In a short experiment at -5° C hedgehogs exhibited continuous respiration (Soivio et al. 1968).

The periodic respiratory pattern seemed to be more regular in the hedgehog than in the garden dormouse. In studies at 4° C Kristoffersson & Soivio (1964b) considered the Cheyne-Stokes respiratory pattern to be 'probably one of the most sensitive criteria of undisturbed hypothermia'. However, in contrast to the garden dormouse, the hedgehog also reacted to experimental disturbances by curling itself into a tighter ball. In garden dormice, for some unknown reason, rising movements were sometimes detectable during periodic respiration, but curling movements occurred only during an induced arousal from deep hibernation. In the hedgehog the change in the respiratory rhythm was found to be the first sign of a spontaneous arousal (Kristoffersson & Soivio 1967), but in contrast to the garden dormouse (Pajunen 1970, 1974), periodic respiration appeared to continue after the first rise in $T_B$. The periodic respiratory pattern was maintained at the beginning of an induced arousal in both animals (Kristoffersson & Soivio 1964b, Pajunen 1970).

In the golden hamster short apnoeas have been registered at 4° C: on average 8 min, with 6-8 respiratory movements between them (Kristoffersson & Soivio 1966). The golden hamster, with hibernation periods of 4.4 days' duration at 4° C, seems to be a poor hibernator compared with the hedgehog and garden dormouse. Hedgehogs hibernated on average for 10-13 days (Kristoffersson & Soivio 1964a). Finnish garden dormice for 11 days (Pajunen 1970), French males for 15 days and females for 12-13 days (Pajunen 1974) at the same $T_A$ in midwinter, when the hibernation periods are of maximal length. Furthermore, mortality in the golden hamster was high during the hibernation season. These animals were provided with food, whereas no food was given to hedgehogs or garden dormice. In a short experiment at 10° C the periodic respiratory pattern was less pronounced in the golden hamster. Several successive respiratory movements were followed by apnoeas of 50-60 s duration (Kristoffersson & Soivio 1966). This $T_A$ appears to be close to the $T_A$ at which the periodic respiratory pattern changes to a continuous one in the golden hamster. In the garden dormouse the periodic respiratory pattern has been observed at relatively high $T_A$s: e.g. in autumn at 16° C before transference to the hibernaculum (Pajunen 1976).

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