EXPERIMENTALLY INDUCED DISRUPTION OF THE DIURNAL RHYTHM OF BODY TEMPERATURE OF THE RAT

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RESUMO

Embora seja sabido que a temperatura corpórea dos mamíferos pode ser afetada temporariamente por perturbações ambientais e pela administração de agentes farmacológicos, pouco se sabe a respeito de perturbações duradouras do ritmo diurno da temperatura corpórea (RDT). No presente estudo, o RDT de ratos Long-Evans permaneceu inalterado sob condições de escuridão contínua bem como após diversos tratamentos experimentais sob ciclo de luz e escuridão. Tratamentos que não afetaram o RDT significativamente incluem confinamento por 24 h, privação alimentar, indução de hipotermia, inalação de éter, e injeção intraperitoneal de quetamina, quetamina/xilazina, etanol, propranolol, ou levedo. Apenas dois tratamentos causaram perturbações duradouras (4-5 dias) do RDT: cirurgia abdominal e administração aguda de paraclorofenilalanina. Um mecanismo comum da ação desses dois tratamentos não foi identificado.

UNITERMOS: ritmo circadiano, temperatura corpórea, rato.

ABSTRACT

Although the body temperature of a mammal is known to be transiently affected by several environmental treatments as well as by the administration of numerous pharmacological agents, very little is known about disruptions of the diurnal rhythm of body temperature (DTR) that last for several days after the experimental treatment. In the present study, the DTR of Long-Evans rats persisted under conditions of constant darkness as well as after a number of experimental treatments under a light-dark cycle. Treatments that did not significantly affect the DTR included 24-hr restraint, food deprivation, environmentally-induced hypothermia, ether inhalation, and intraperitoneal injections of ketamine, ketamine/xylazine, ethanol, propranolol, or brewer's yeast. Only two treatments caused a long-lasting (4-5 days) disruption of the DTR: abdominal surgery and acute administration of parachlorophenylalanine. No common mechanism for the action of these two treatments was identified.

KEY WORDS: circadian rhythm, body temperature, rat.

Introduction

A diurnal oscilation in the body temperature of the rat, consisting of an approximately sinusoidal curve with a nocturnal peak and a diurnal trough, was described over 30 years ago (Halberg **et al.**, 1954; Heusner, 1959). Although numerous studies have examined acute changes in body temperature in response to environmental manipulation (Georgiev, 1978; Morrion and Ryser, 1959; Poole and Stephenson, 1977) and to injection of foreign substances (Gordon **et al.**, 1988; Myers, 1984), little attention has been directed at disruptions of the diurnal rhythm of body temperature (DTR) for periods of days or weeks. The process of aging seems to cause an irreversible flattening of the DTR (Sacher and Duffy, 1978; Halberg **et al.**, 1981), and intraperitoneal injections of parachlorophenilalanine (PCPA, a serotonergic synthesis inhibitor) causes a flattening of the DTR that lasts 3-4 days in pigeons and rats (Necker and Wegner, 1981; Tomkowiak **et al.**,

1988). The present study was conducted to investigate the effect of various experimental treatments on the DTR of the rat.

Materials and Methods

Male and female Long-Evans rats were housed individually in plastic cages (20 x 25 x 21 cm) at 23°C and fed Purina lab chow and water ad libitum. A 12:12 hr L:D cycle was maintained throughout (lights on from 0800 to 2000 hr), except if otherwise stated.

At the beginning of the experiment, the rats were anesthetised with a mixture of ketamine and xylazine (80 and 12 mg/kg, respectively, i.p.), implanted with a temperature transmitter model M (Mini-Mitter Company, Sunriver, Oregon), and placed in the individual cage inside sound proof chambers. An AM radio receiver sat next to each animal cage and was interfaced to a microcomputer. The implanted transmitter continually emited a series of AM signals, the frequency of which was proportional to the temperature of the transmitter. At 10-min intervals, a microcomputer measured the frequency of the AM signals and calculated the temperature in accordance with a calibration curve obtained before the transmitter had been implanted.

Besides the surgery for implantation of the transmitter, groups of six rats were subjected to one or more of 11 experimental treatments: 1) constant darkness instead of a 12:12 L:D illumination regime, 2) injection of a mixture of ketamine and xylazine (80 and 12 mg/kg, respectively, i.p.) without subsequent surgery, 3) injection of ketamine HCL (110 mg/kg, i.p.), 4) forced inhalation of ether until loss of muscle tone, 5) injection of brewer's yeast (75 mg/kg, i.p.) to induce a fever, 6) injection of PCPA (300 mg/kg, i.p.), 7) injection of ethyl alcohol (3 g/kg, i.p.), 8) injection of

of the β-adrenergic blocker propranolol (10 mg/kg, i.p.), 9) induction of acute hypothermia (body temperature of 32°C) by restraint in a Plexiglas tube and immersion in a 5°C water bath for 5-10 min, 10) 24-hr food and water deprivation, and 11) 24-hr restraint in a Plexiglas tube (placed inside the home cage) together with food and water deprivation. During or immediately after each of these treatments, the animals were kept in their home cages and their body temperature was recorded continuosly.

The overall effect of the experimental treatments was tested by a one-way ANOVA. Differences between pairs of treatments were tested by Tukey's HSD test.

Results

The DTR of a normal rat entrained to a 12:12 hr L:D cycle is very consistent from day to day, as exemplified in Fig. 1. The mean amplitude of the daily cycle (absolute peak minus trough) of 30 control rats was 1.83 ± 0.12°C.

Maintaining the rats under constant dark (DD) rather than under a 12:12 hr regime had no significant effect on the amplitude of the DTR, although the period of the rhythm became slightly longer as the animals freeran. An example of a rat kept in DD for 25 days is shown in Fig. 2. Mean amplitude was 1.76 ± 0.07°C under LD and 1.70 ± 0.05 under DD, the difference not being statistically significant: $t(24) = 0.62$, $p > 0.10$.

Body temperature records of four rats beginning immediately after surgery are shown in Fig. 3. In a few hours, all animals recovered from the acute hypothermia induced by the anesthetic. However, the DTR was almost flat for 3-4 days after surgery, which indicates that postoperatory recovery is not complete for several days.

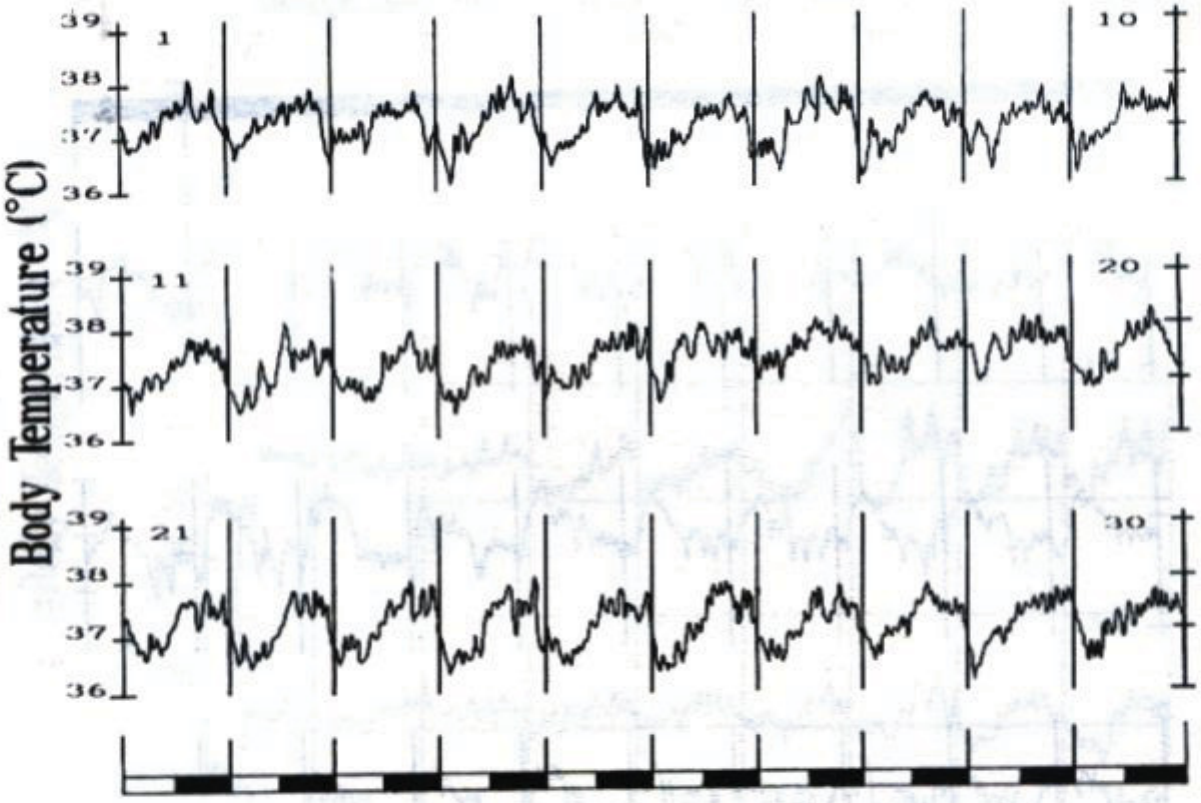

Fig. 1 - Records of the body temperature of a rat kept under LD 12:12 for 30 consecutive days.

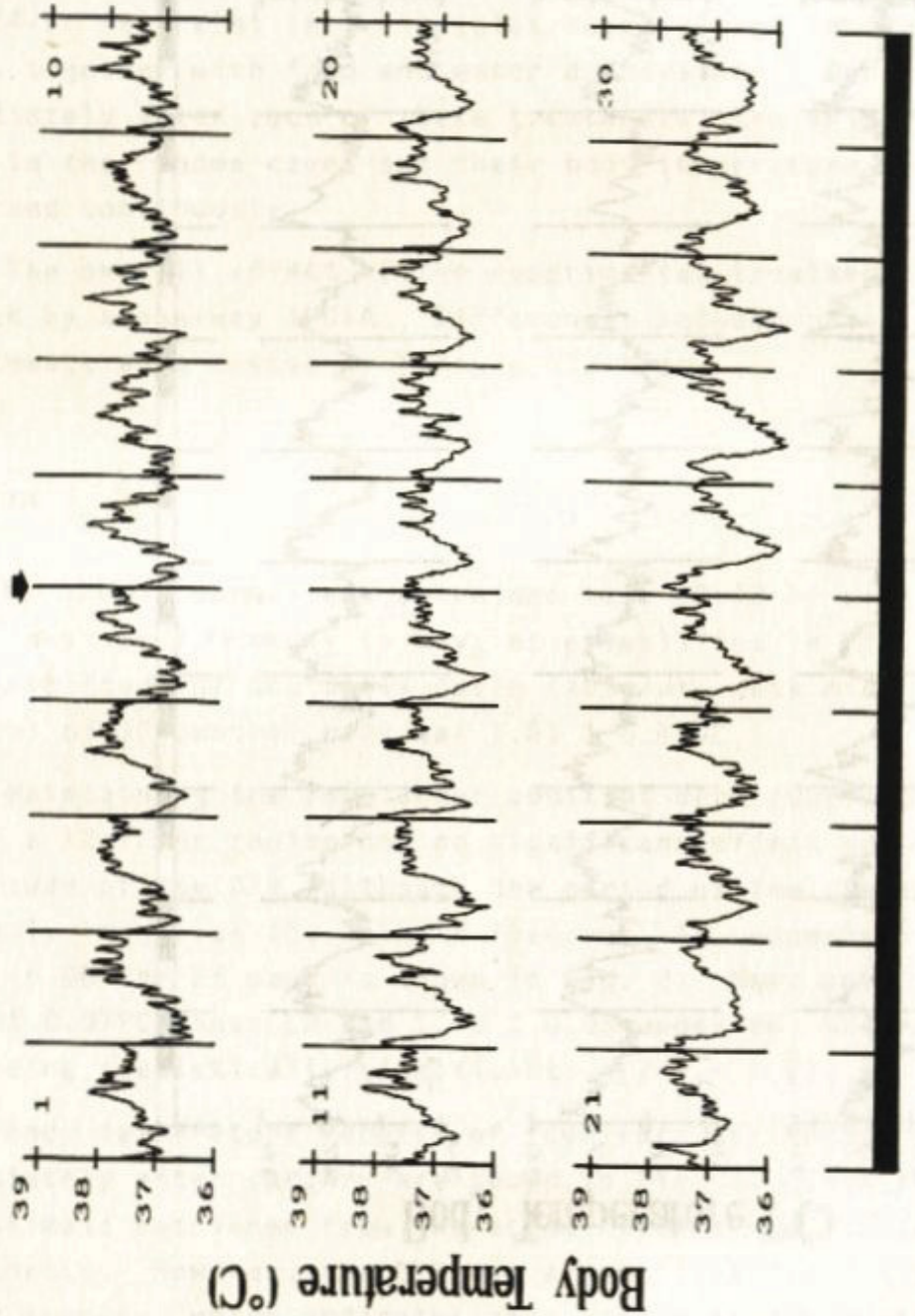

Fig. 2 - Records of the body temperature of a rat kept in constant dark for 25 days after 5 days of LD 12:12 (arrow).

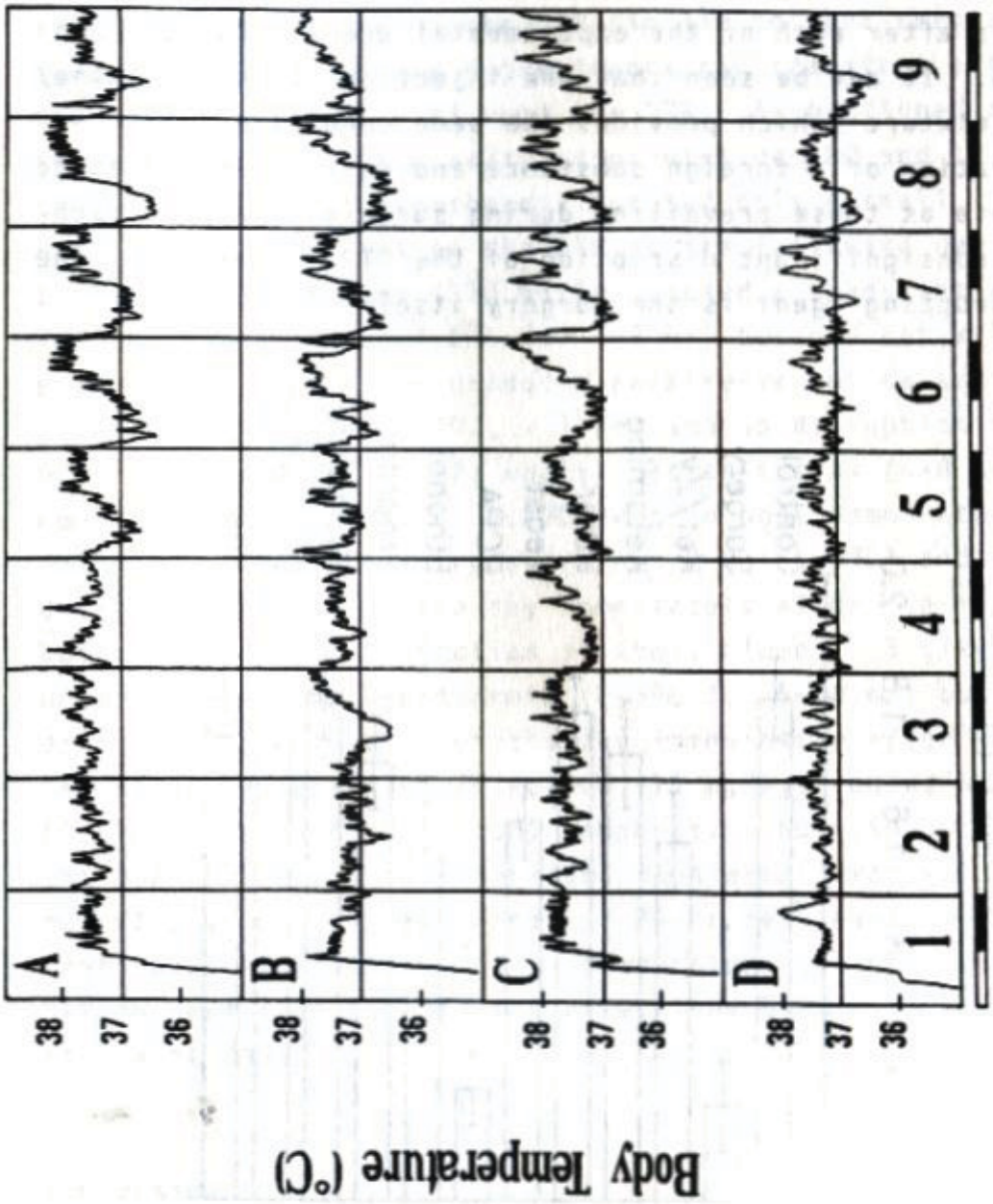

Fig. 3 - Records of the body temperature of four rats kept under LD 12:12 for 9 days. Surgery for implantation of the temperature transmitter was conducted on Day 1.

Because a typical surgery includes not only the abdominal incision and suture but also the administration of a foreign substance (the anesthetic) and concomitant hypothermia, the data in Fig. 3 do not allow a conclusion about the agent causing the loss of the DTR. The mean DTR amplitude for the three days after each of the experimental treatments is shown in Fig. 4. It can be seen that the injection of the ketamine/xylazine mixture (which provides the same conditions of administration of a foreign substance and development of acute hypothermia as those prevailing during surgery) caused a much smaller, nonsignificant disruption of the DTR. Therefore, the major disrupting agent is the surgery itself.

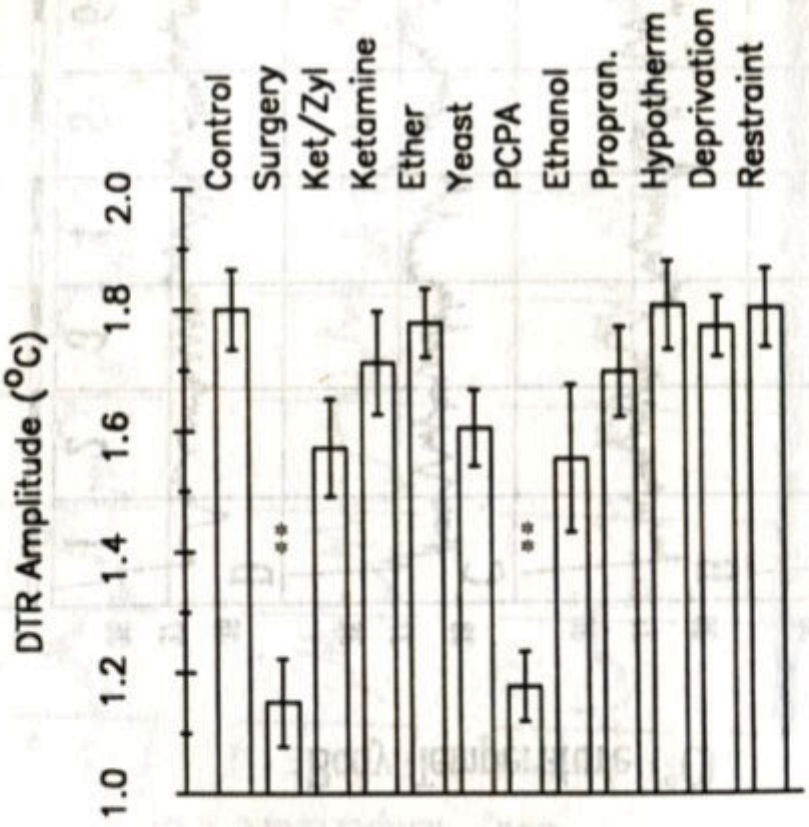

Fig. 4 - Mean DTR amplitude for the 3 days following the day of experimental treatment. Each bar refers to the mean (± SE) of six rats for each treatment. Double stars indicate significant difference from control group ($p < 0.01$).

Of the 11 treatments shown in Fig. 4, only two had a significant effect on the amplitude of the DTR: surgery and injection of PCPA (300 mg/kg). PCPA caused a strong transient drecrease in body temperature (3-4°C for 4-6 hr) and a 3-4 day flattening of the DTR similar to that seen after surgery. The remaining 9 treatments had transient effects but did not significantly disrupt the DTR. As mentioned earlier, injection of the ketamine/zylazine mixture (80 and 12 mg/kg) caused a transient hypothermia but had only a small, nonsignificant effect on the DTR in the following days. Injection of ketamine (110 mg/kg) caused a transient elevation in body temperature (1-2°C for 1-2 hr) but did not affect the DTR. Ether inhalation produced anesthesia but caused only a brief hyperthermia (< 1°C, < 1 hr) and no disruption of the DTR. Yeast injection (75 mg/kg) caused a slow (6-8 hr) fever consisting of an initial 0.8°C drop in body temperature followed by a 1.8°C increase (i.e., 0.8 + 1.0°C) and disrupted the DTR on the day immediately after the injection but not later on. Injection of ethyl alcohol (3 g/kg) produced a strong hypothermia (2-3°C for 5-10 hr) but disrupted the DTR only on the day immediately after the injection. Propranolol injection (10 mg/kg) caused a brief (0.5°C, < 1 hr) drop in body temperature but did not affect the DTR. Forced hypothermia and food/water deprivation had no effects on the DTR. Finally, 24-hr restraint caused a continuous elevation cf body temperature (which masked the DTR on that day) but did not affect the DTR in the following days.

Discussion

The results indicate that the diurnal rhythm of body temperature of the rat is a robust biological rhythm that persists in the absence of a light-dark cycle and is not

disrupted, except transiently, by several forms of stress. Although initial observations on the DTR of the rat suggested that the rhythm was not endogenously generated (Fioretti **et al.**, 1974), many researchers have since demonstrated the persistence of the DTR for at least several months in conditions of constant light (Eastman and Rechtschaffen, 1983; Honma and Hiroshige, 1978) or constant dark (Satinoff **et al.**, 1982; Satinoff and Prosser, 1988).

Two of the experimental treatments produced a significant flattening of the DTR that lasted several days. The effect of sugery was not due to the effect of the anesthetic used during surgery, as the anesthetic alone (ketamine/xylazine mixture) caused a much smaller disruption of the DTR. Since ketamine alone did not disrupt the DTR, the effect of the ketamine/xylazine mixture must have been due to xylazine. The major effect, however, was derived from the surgery itself, either because of the incision and suture or because of the insertion of a foreign body (the temperature transmitter).

Of the 7 pharmacological agents that were tested, only PCPA had a strong disruptive effect on the DTR for several days after the injection. This effect has been observed before in pigeons (Necker and Wegner, 1981) and rats (Tomkowiak **et al.**, 1988). PCPA is a serotonin synthesis inhibitor that acts by inhibiting the action of tryptophan hydroxylase (Maickel, 1986). As the synthesis of melatonin utilizes serotonin, PCPA inhibits also melatonin synthesis. Melatonin has been implicated in the control of circadian rhythms (Chesworth **et al.**, 1987), and the inhibition of melatonin by PCPA might explain the flattening of the DTR. Further studies are necessary to elucidate this question.

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