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The time of day differently influences fatigue and locomotor activity: Is body temperature a key factor?

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HIGHLIGHTS

- Each kind of physical activity is distinctively influenced by the 24 h cycle.
- Running capacity is higher during the light phase of the daily cycle.
- Locomotor activity is higher during the dark phase of the daily cycle.
- Exercise performance and locomotor activity are not directly associated.
- Thermal balance is influenced by the time of day.

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ABSTRACT

The aim of this study was to verify the possible interactions between exercise capacity and spontaneous locomotor activity (SLA) during the oscillation of core body temperature (T_b) that occurs during the light/dark cycle. Wistar rats ($n = 11$) were kept at an animal facility under a light/dark cycle of 14/10 h at an ambient temperature of 23 °C and water and food ad libitum. Initially, in order to characterize the daily oscillation in SLA and T_b of the rats, these parameters were continuously recorded for 24 h using an implantable telemetric sensor (G2 E-Mitter). The animals were randomly assigned to two progressive exercise test protocols until fatigue during the beginning of light and dark-phases. Fatigue was defined as the moment rats could not keep pace with the treadmill. We assessed the time to fatigue, workload and T_b changes induced by exercise. Each test was separated by 3 days. Our results showed that exercise capacity and heat storage were higher during the light-phase ($p < 0.05$). In contrast, we observed that both SLA and T_b were higher during the dark-phase ($p < 0.01$). Notably, the correlation analysis between the amount of SLA and the running capacity observed at each phase of the daily cycle revealed that, regardless of the time of the day, both types of locomotor physical activity have an important inherent component ($r = 0.864$ and $r = 0.784$, respectively, $p < 0.01$) without a direct relationship between them. This finding provides further support for the existence of specific control mechanisms for each type of physical activity. In conclusion, our data indicate that the relationship between the body temperature and different types of physical activity might be affected by the light/dark cycle. These results mean that, although exercise performance and spontaneous locomotor activity are not directly associated, both are strongly influenced by daily cycles of light and dark.

1. Introduction

Daily oscillations in heat production have been associated with metabolic, cardiovascular, endocrine and central nervous system (CNS) functions [1]. In addition, it is well known that running capacity relies on body internal heat balance, substrate availability and cardiovascular function. As these parameters are also influenced by the endogenous rhythms, intrinsic daily rhythmic oscillations in heat production might be related to exercise capacity.

The CNS primarily controls circadian rhythms through the hypothalamic suprachiasmatic nucleus (SCN) and its afferent and efferent projections [2]. In general, the circadian rhythms are mediated by the activity of the neuroendocrine system and the autonomic nervous system [3,4], which directly impacts core body temperature (T_b), spontaneous locomotor activity (SLA) and heart rate [5]. The circadian T_b and SLA are central parameters to daily energy balance [6,7]. In line with an increased metabolic demand, animals with nocturnal habits show peaks

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of T_b and SLA during the early hours of environmental darkness. By contrast, the lowest points are observed shortly after the light-phase onset and are usually associated with a lower metabolic demand during the inactivity period. Although being closely associated to heart rate and SLA (i.e., foraging, alertness, and feeding), T_b shows a SCN-dependent rhythmic component [8] independent of SLA [9]. In fact, changes in the autonomic balance across the day might help to understand how these daily rhythms interact to maintain *homeostasis*, even under potentially distinct stressful situations, regardless of time of the day [3,10].

Thermal balance is achieved by regulation of heat production and dissipation mechanisms [11,12]. Physical exercise induces an acute temporary imbalance in thermoregulatory capacity. The initial raise in T_b is promptly followed by the activation of heat loss mechanisms to keep heat storage at a safe level for the organism [11]. The choice to stop running is most likely due to the inability to keep the internal balance, which results from an increased body heating rate without a higher heat dissipation rate [13,14] or a critical core temperature [15,16]. Fatigue results, and this leads to exercise interruption. In fact, thermoregulatory mechanisms and exercise capacity share common central pathways directly related to autonomic imbalance [17–21]. Therefore, circadian rhythms and exercise performance regulation might share common regulatory pathways.

As exercise capacity is directly influenced by body temperature [11], a daily oscillation in exercise performance could be hypothesized. Recently, a reciprocal influence in physical performance and daily rhythms has been investigated both in animals and in humans. Morning and afternoon muscle power, function and contractility are consistently different across the day under cool and warm environments [22]. An internal clock mediating maximal aerobic exercise capacity has been suggested [23] and thermoregulation has been noted as a possible bond for the predicted influence of circadian agents in exercise capacity [24]. In rats, heat balance during low-to-moderate intensity treadmill exercise is affected by the time of day [25]. Moreover, environmental light conditions seem to induce changes in thermoregulation at rest and at low intensity exercise with a possible influence of the time of day [26]. In addition, the effect of the time of day on maximal exercise capacity and thermoregulatory capacity has been less explored.

To verify whether performance- and exercise-induced thermoregulatory changes are influenced by the time of day, adult rats were subjected to exercise until fatigue on a motor-driven treadmill at the onset of the light and dark-phase of the light/dark cycle. In addition, the 24 h oscillation of core body temperature and spontaneous locomotor activity were registered to analyze whether those patterns were associated with the differences found between exercise capacity and thermoregulatory parameters at the early stages of the active and inactive periods of the luminosity cycle.

2. Methods

2.1. Animals

Adult male Wistar rats (n = 11) with an average body weight of 297 ± 5 g were individually housed in a room with a cycle of 10 h of exposure to dark and 14 h of exposure to light, with the lights being turned on at 06:00 (defined as *zeitgeber* time 0, ZT0) and turned off at 20:00 (ZT14). Housing conditions included ad libitum water and chow (NUVILAB-CRI, PR, Brazil) and a constant ambient temperature of 23 °C.

All of the described experimental procedures were approved by the local ethics committee for animal experimentation (CETEA/UFMG) under the protocol number 139/2008 and followed the APS Resource Book for the Design of Animal Exercise Protocols.

2.2. Surgical procedures

Animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (11.6 mg of 10% ketamine for 100 g of animal body weight) and xylazine (0.57 mg of 2% xylazine for 100 g of animal body weight). A ventral incision at the linea alba was made to introduce a telemetric sensor (G2 E-Mitter, Mini-Mitter Company, Sun River, OR, USA) into the peritoneal cavity. Each probe was then sutured to the inner musculature before the incision was closed. This procedure allowed continuous monitoring of both T_b and SLA with a decreased risk of internal displacement of the sensor, which could cause misleading readings due to its position. At the end of the surgery, a single dose of *intramuscular* 24,000 U/kg of procaine penicillin (Pentabiótico Veterinário ®, Fort Dodge Animal Health Ltda, Jaguariuna, SP, Brazil) and *subcutaneous* 1.1 mg/kg of non-steroidal anti-inflammatory analgesic (Banamine ®, Scering-Plough, São Paulo, SP, Brazil) were administered. After this procedure, the animals were allowed to recover for 3 days before beginning the recording of the daily cycle of SLA and T_b.

2.3. Recordings of 24 h of T_b and SLA

On the first day of the experiment, animals were placed inside individual cages situated over a telemetry signal receptor (ER-4000 Energizer/Receiver, Mini-Mitter Company, Sun River, OR, USA) that was previously configured to catch the specific signal frequency emitted by the sensor probe implanted into the peritoneal cavity. The data were transmitted to a computer with VitalView Software (VitalView ® Data Acquisition System Software v. 4.0, Mini-Mitter Company, Sun River, OR, USA) and stored. For 24 h, the animals were kept under these conditions to evaluate a single daily oscillation of SLA and T_b.

Each animal was individually housed inside a standard cage in a calm and separated room with the photoperiod set at 14 h of artificial light (lights on at 06:00) followed by 10 h of darkness (lights off at 20:00) and a controlled ambient temperature (23 °C). Water and food (standard rat chow, NUVILAB, São Paulo, SP, Brazil) were provided ad libitum. To avoid handling influence by the experimenter, only the data generated 12 h after the recording began was analyzed.

T_b (°C) and SLA (arbitrary units/min) were continuously recorded every minute of a 24-hour period. Both of these parameters were averaged for further analysis. The means for each phase of the photoperiod were calculated for comparison and to confirm that the experimental arrangement was in agreement with the nocturnal habits described for Wistar rats. Hourly means were also calculated to confirm the oscillation of T_b and SLA [27]. In addition, these averages were used to further study the correlation between these two parameters throughout the day and with exercise capacity.

2.4. Treadmill exercise protocols

To test our hypotheses that physical performance is influenced by the light/dark cycle and that this difference is influenced by daily T_b and SLA oscillations, exercise capacity and T_b were measured on separate days at the early daytime and nighttime hour during incremental exercise tests until fatigue, and the data were compared.

On the first day following the previous experiment, animals were acclimated to a motor-driven treadmill adapted for small rodents (GAUSTEC Magnetism, Contagem, MG, Brazil). This familiarization process took 5 days and consisted of a running activity of 5 min with a speed ranging from 10 to 15 m/min and a constant slope of 5°. A slight electrical stimulation (0.4 mA) was provided to assure that animals were capable of exercising. This procedure was necessary so that the animals could learn the orientation of the running activity and reduce the influence of new environmental conditions in the exercise-related responses without any major adaptations induced by exercise training.

At the beginning of the third week of experiments, maximal exercise capacity was evaluated during the early stages of the light and dark-phase. Each test was separated by 3 days. The T_b was assessed before the onset of exercise and after exercise-induced fatigue. After the last experimental session, the animals were euthanized by anesthetic overdose.

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