RESEARCH PAPER

Independent effects of rapid eye movement sleep deprivation and exposure to environmental heat stress on aerobic performance and thermoregulatory responses in exercising rats

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ABSTRACT

Evidence indicates that aerobic performance is degraded either by environmental heat stress or sleep deprivation. However, whether these conditions interact to produce more significant performance impairment deserves further investigation. Therefore, this study investigated the effects of experimental sleep deprivation (24 h or 96 h) on aerobic performance and thermoregulatory responses in rats exercised on a treadmill at different environmental conditions. Adult male Wistar rats were subjected to rapid eye movement sleep deprivation (RSD) using the modified multiple platform method and were then subjected to an incremental-speed exercise until they were fatigued. Treadmill running was performed in a temperate (24°C) or warm (31°C) environment, and the colonic temperature (an index of core body temperature; T_{CORE}) and the tail-skin temperature (T_{SKIN} ; an index of cutaneous heat loss) were recorded. 24-h and 96-h RSD produced small magnitude reductions in aerobic performance (Cohen's $d = 0.47$ – 0.58) and minor changes in thermoregulation. Relative to control rats, sleep-deprived rats showed a higher T_{CORE} at the exercise initiation and a higher threshold for activating cutaneous heat loss, but unchanged T_{CORE} and T_{SKIN} at fatigue. Exercise at 31°C induced large reductions in performance ($d = 0.82$ – 1.29) and marked changes in thermoregulation, as evidenced by higher T_{CORE} and T_{SKIN} at fatigue, compared to exercise at 24°C. Interestingly, none of the effects induced by RSD were exacerbated by environmental heat stress and vice-versa, indicating that both conditions did not interact. We conclude that RSD and heat stress modulate aerobic performance and thermoregulatory responses by acting independently.

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Introduction

Sleep deprivation is a common issue in modern societies [1], and its prevalence has been increasing due to constant exposure to artificial light and interactive activities, which combine with social and economic pressures to shorten the time spent asleep [2]. Sleep deprivation can be divided into conditions of complete (such as in ultra-endurance sporting events and military exercises) or partial deprivation (such as in individuals experiencing sleep disorders or flying across time zones, and in shift workers) [3]. Affected individuals usually report feelings of persistent tiredness and lack of energy [2,3].

In the sports context, sleep disruption in athletes often occurs due to rapid travel across

multiple meridians, anxiety before important events, and training and competition schedules [4–6]. A study with a large number of nationally-ranked athletes revealed they only sleep between 6 and 7 h per night [7]. Evidence suggests that reduced sleep is associated with altered neurotransmission [8,9] and neurobehavioral deficits in daytime performance [7]; therefore, the level of sleep loss commonly observed in athletes could be associated with impaired performance and recovery.

Previous studies have reported reduced aerobic performance following total sleep deprivation in humans [10–12], although this is not a universal observation [13]. Because aerobic performance is modulated by thermoregulation, there are investigations about the impact of sleep deprivation on

body temperatures in individuals subjected to physical exercise. A lower core body temperature (T_{CORE}) was observed during exercise in sleep-deprived subjects [12], even though a tendency for enhanced exercise-induced increases in T_{CORE} was also reported [14]. Noteworthy, most of the studies on the topic have failed to notice differences in the increase in T_{CORE} induced by cycling [15,16] or running [17].

In the laboratory, experimental models have been developed for studying the consequences of sleep deprivation in rats. This study will focus on repeated eye movement (REM) sleep, which is assumed to play a role in restoring some deficits incurred during prior wakefulness and preparing for ensuing wakefulness [18]. In this sense, REM sleep deprivation (RSD) adversely affects several body functions, including food intake, energy metabolism, body weight [19], and the regulation of T_{CORE} . Several authors demonstrated that total sleep deprivation and RSD modify the thermoregulatory responses in rats, with either report of hypothermia [20–24] or hyperthermia [20,21,23–25]. Of note, the predominant thermoregulatory manifestation depends on the duration of sleep deprivation: the initial hyperthermia is usually replaced by hypothermia as deprivation progresses and the risk of dying increases [20,21,23,24]. Because increased T_{CORE} at exercise initiation reduces aerobic performance, particularly in the heat [26,27], rats subjected to short-term RSD (i.e., 24 h or 96 h) would likely fatigue earlier than control rats.

Like sleep deprivation, warm/hot environments impact aerobic performance and thermoregulatory responses during prolonged exercise [26,28,29]. For example, rats exhibit greater increases in brain, abdominal, and tail-skin (T_{SKIN}) temperatures [26,30,31], greater cardiovascular strain [32,33], and reduced aerobic performance [26,30–32] while exercising in warm/hot conditions compared with temperate conditions. Running in the heat also changes neurotransmission in rats, as indicated by higher dopamine and noradrenaline concentrations in the preoptic area and anterior hypothalamus [34]. Therefore, because RSD and environmental heat stress influence neurotransmission, it is likely that the performance and thermoregulatory impairments induced by these two

factors share common pathways, so that the effects mediated by RSD would be exacerbated in a warm environment.

Thus, the present study aimed to evaluate the effects of either 24-h or 96-h RSD on aerobic performance and thermoregulatory responses in rats subjected to an exercise session at different environmental conditions. Considering that T_{CORE} levels at exercise initiation modulate aerobic performance, we hypothesized that sleep-deprived rats would exhibit higher initial T_{CORE} and altered thermoregulatory control during exercise that would accelerate fatigue. We also expected that the thermoregulatory and performance impairments would be more evident in sleep-deprived rats under environmental heat stress conditions.

Materials and methods

Animals

Adult male Wistar rats weighing 250–350 g were used in the experiments. The rats were housed in collective polypropylene cages (3 animals per cage), under controlled light (lights on from 7:00 am until 7:00 pm) and temperature ($24 \pm 1^\circ\text{C}$) conditions, with water and rat chow provided *ad libitum*. All experimental procedures were approved by the local ethics commission for the use of animals (protocol 273/2014) and were performed following the regulations provided by the National Council for the Control of Animal Experimentation (CONCEA/Brazil).

Experimental design

The study was divided into four experiments (Figure 1), and different rats were used in each experiment. The first experiment aimed to investigate the effects of a 24-h RSD on thermoregulatory responses and aerobic performance of rats subjected to an incremental-speed fatiguing exercise in a temperate environment (24°C). Moreover, the experiment 1 also aimed to investigate whether our control conditions (rats kept on a grid where they could sleep) represented an adequate control trial indeed. Therefore, the thermoregulation and performance of rats kept on the grid (without any contact with cedar-wood shavings) were compared

Exp 1: 24-h RSD vs. controls at 24°C **Exp 2: 24-h RSD vs. controls at 31°C** **Exp 3 and 4: 96-h RSD vs. controls at 24°C or 31°C**

Figure 1. Experimental designs. * indicates the days at which the incremental-speed exercises were carried out. Legend: Exp, experiment; RSD, rapid eye movement sleep deprivation.

to the responses of rats kept in their home cages filled with cedar-wood shavings. In this first set of experiments, nine rats were subjected to three trials: (I) home cage-control – rats that were not subjected to the RSD protocol and that could sleep in their home cages on cedar-wood shavings; (II) control – rats that were not subjected to the RSD protocol and that could sleep on the grid; (III) sleep deprivation – rats that were subjected to the RSD protocol. This protocol (or the control procedure) was always initiated at 7:00 am and ended at 7:00, 8:00, or 9:00 pm on the same day, when the exercise session was initiated. A minimum interval of 72 h separated the exercise sessions, and the order of the trials was balanced, as defined by a Latin square design.

The second experiment investigated the effects of 24-h RSD on thermoregulation and performance of rats subjected to incremental exercises in a warm environment (31°C). Nine animals were subjected to two trials: (I) control and (II) sleep deprivation. During the experimental trials, the procedures were similar to those adopted in the first set of experiments, except for the ambient temperature and the inexistence of a control trial with rats kept in their home cages. The order of the trials was randomized.

The third experiment investigated the effects of 96-h RSD on thermoregulation and performance of rats subjected to incremental exercises in a temperate environment. Nine rats were subjected to two trials (i.e., control and sleep deprivation). The RSD protocol was always initiated at 7:00 am

and ended at 7:00, 8:00, or 9:00 pm on the fourth day, when the exercise sessions were initiated. A minimum interval of 120 h separated the exercise sessions, and the order of the trials was randomized. The fourth experiment was identical to experiment 3, except the ambient temperature at which treadmill running was carried out (i.e., 31°C, warm environment).

In all experiments, exercise was performed at the transition between the light (inactive) and dark (active) phases when rats are expected to wake up. We also avoided subjecting the same rat to two RSD procedures, because as the animals are subjected to more days into a sleep-deprived state, they became more adapted to this condition, possibly changing their sleep profile over time [35]. Moreover, following the last trial, the rats were euthanized with an intraperitoneal injection of a lethal dose of anesthetic (ketamine 240 mg/kg and xylazine 31.5 mg/kg).

Familiarization with the experimental procedures

The familiarization protocol was conducted across five consecutive days. Light electrical stimulation (0.5 mA) was applied to gradually encourage the rats to exercise on a treadmill designed for small animals (Panlab, Harvard Apparatus, Spain). In each daily session, after resting for 5 min on the treadmill belt, the rats were required to run for 5 min at a constant speed (11 m/min on the first day, with a daily increase of 1-m/min) and a constant incline of 5%. In the last three familiarization sessions, the rats run with a thermistor

inserted into their colon and a thermocouple attached to the lateral surface close to the base of their tail. The purpose of these preliminary exercise sessions was to show the rats the direction they should run without becoming entangled in the thermistor/thermocouple wires, thereby allowing for consistent treadmill performance and minimizing their exposure to electrical stimuli during the experimental trials [36,37]. Animals were also familiarized with the sleep deprivation procedure for 60 min during these five consecutive days.

RSD protocol

Sleep deprivation was accomplished by using the modified multiple platform method proposed by Suchecki [38]. This method is known as an inducer of RSD (or paradoxical sleep deprivation, as commonly used in the past), although it also produces partial deprivation of slow-wave sleep [39]. Seven platforms (6 cm in diameter each) were placed inside a big polypropylene cage (56 x 36 x 21 cm) filled with water. Three rats, always taken from the same home cage in the vivarium, were placed in a cage with multiple platforms to prevent social instability from enhancing stress [38]. The rats were placed on top of the platforms so that they could move around. The cages were filled with water until 1 cm below the top of the platforms, and the water was changed daily. Because of atonia in the antigravity muscles at the onset of REM sleep, the rats on the small platforms tended to fall into or touch the surrounding water, resulting in REM sleep loss [24]. Exposure to the multiple platforms began at 7:00 am and lasted for 12 h or 84 h.

Control experiments were performed in the same big polypropylene cages placed in the same room as the sleep-deprived rats for the duration of the procedure. However, the control rats were placed on a metallic grid floor, with water until 1 cm below the floor. The rats were allowed to lie down without falling into the water, albeit their tails might have touched the water [38]. The grid was made of stainless steel, with the rods placed 2.3 cm apart from each other.

The room where RSD and control protocols were carried out was maintained under controlled light (lights on from 7:00 am until 7:00 pm) and

temperature ($24 \pm 1^\circ\text{C}$) conditions, which were similar to the conditions maintained at the vivarium. Food and water were provided *ad libitum*.

Physical exercise trials

The rats were always subjected to the exercise trials at 7:00, 8:00, or 9:00 pm (beginning of the dark phase). On the day when experiments were conducted, each rat was weighed, a thermistor (MEAS 4400, Dayton, OH, USA) was inserted 7 cm beyond the anal sphincter to measure colonic temperature (an index of T_{CORE}), and a thermocouple was attached to the lateral surface, 1 cm from the base of the tail (Instrutherm, model S-09 K, Brazil) to measure T_{SKIN} . The close proximity to the base of the tail enabled more sensitive measurements of changes in T_{SKIN} that occurred as a function of changes in local blood flow [40]. Notably, the T_{SKIN} in heat-exchange organs, such as the rat tail, primarily depends on the local vasomotor tone and is not a reliable measure of ambient temperature [41].

The rats were then subjected to an incremental speed exercise (starting at a speed of 13 m/min, with 1.3 m/min increases every 3 min). The exercise was performed until volitional fatigue, which was defined as when the rats were no longer able to keep pace with the treadmill for 10 s, even when stimulated by light electrical shocks [36,42].

The T_{CORE} and T_{SKIN} were recorded every 60 s throughout the exercise sessions, and the time to fatigue was considered a physical (aerobic) performance index.

Ambient temperature control

Ambient temperature (T_{AMB}) was measured inside the chamber that involves the treadmill belt with two thermocouples, one positioned in the front and the other in the back of the treadmill. In the experiments performed under temperate conditions, T_{AMB} was controlled at 24°C with air-conditioning. This T_{AMB} was selected as a temperate environment because previous data suggest that T_{AMB} ranging from 24 to 26°C corresponds to the lower extremity of the thermoneutral zone of resting rats maintained inside the chamber that contained the treadmill belt

[37,43,44]. In the warm experiments, the T_{AMB} was controlled at 31°C by using two heaters turned on at 1,200 W (Britânia model AB 1100, Curitiba, Brazil), one positioned in the front and the other in the back of the treadmill, in order to avoid the surging of a temperature gradient in the chamber. T_{AMB} was recorded every 60 s throughout the exercise sessions.

Calculations

To identify differences in the central modulation of thermoeffector activity, the T_{CORE} threshold for cutaneous heat loss was calculated using a method based on that described by Cheuvront et al. [45]. This method has been used to investigate thermoregulatory responses in rats [31,37]. Briefly, the T_{SKIN} was plotted against T_{CORE} , and then two independent investigators identified the threshold for activation of tail heat loss visually; a consensus-derived value was used. Next, the data before and after this threshold were separated. Linear regression analyses were performed for data before and after the threshold to describe the relationship between T_{SKIN} and T_{CORE} . The intersection of the regression lines was used to determine the heat loss threshold. At this moment, we could objectively verify whether visual identification of the threshold was correct. If not, the analysis was redone from the beginning.

Statistical analysis

Shapiro-Wilk's and Levene's tests were used to assess the normality and homoscedasticity of the data. Because all data presented a normal distribution, they were expressed as means $\pm$ standard errors of the mean (SEM). In experiment 1, T_{CORE} and T_{SKIN} were compared across exercise time points and between experimental trials (home cage vs. grid) using two-way ANOVAs with repeated measures. When significant main effects or interactions were observed, these ANOVAs were followed by Tukey's *post hoc* tests to identify differences between pairs of means. The time to fatigue, T_{CORE} , and T_{SKIN} at exercise initiation and fatigue, exercise-induced changes in T_{CORE} , and the threshold for cutaneous heat loss were compared between trials using paired Student's t-tests.

In the other experiments, T_{CORE} and T_{SKIN} were compared across exercise time points and between treatments (RSD vs. control) and T_{AMB} (24°C vs. 31°C) using three-way ANOVAs. Moreover, the seven parameters mentioned above were compared between treatments and T_{AMB} using two-way ANOVAs, followed by Tukey's tests.

The significance level was set at $\alpha < 0.05$. Cohen's d effect size (ES) was calculated to assess the magnitude of the differences in data between groups. The ES was calculated by subtracting the mean value for one group from the mean value of the group it was being compared to. The result was then divided by a combined standard deviation for the data. The ES values were classified as trivial (< 0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0), very large (2.0–4.0), and extremely large (> 4.0) [46].

Results

Selection of the setup for experiments in control rats

The rats that were kept in their home cages ran for 41.6 ± 2.6 min, which means that on average, they almost have completed the 14th stage of the incremental exercise performed at 24°C. When the rats were kept on the grid (but not subjected to RSD), they displayed similar physical performance relative to the trial when they were kept in their home cages (Table 1).

The rats had similar T_{CORE} at the beginning of exercise, irrespective of whether they were kept (Table 1). Treadmill running markedly increased T_{CORE} in the two experimental trials, so that rats fatigued with temperature values above 40.0°C (Supplementary Figure 1A). Although a trial $\times$ time point interaction was observed, the *post hoc* test failed to identify differences between pairs of means. Moreover, no differences in T_{CORE} were observed between trials at fatigue (Table 1).

At the beginning of exercise, the rats had lower T_{SKIN} when kept on the grid than when kept in their home cages (Table 1); the difference between trials corresponded to a very large effect size. In both trials, T_{SKIN} transiently decreased in response to exercise initiation until reaching a nadir at the 4th min of running (Supplementary Figure 1B).

Table 1. Physical performance and thermoregulatory responses in control rats either kept in their home cages or on the grid and then subjected to an incremental exercise.

	Home cage	Grid	<i>p</i> -value	ES
Time to fatigue (min)	41.6 ± 2.6	43.6 ± 2.5	0.191	0.26
Initial T_{CORE} (°C)	37.87 ± 0.08	38.00 ± 0.08	0.287	0.52
T_{CORE} at fatigue (°C)	40.28 ± 0.25	40.41 ± 0.17	0.458	0.20
Initial T_{SKIN} (°C)	30.81 ± 0.24	28.24 ± 0.30	< 0.001	3.17
T_{SKIN} at fatigue (°C)	33.31 ± 0.35	33.38 ± 0.29	0.896	0.07

Legend: T_{CORE} = core body temperature, T_{SKIN} = tail-skin temperature. Data are expressed as means ± SEM. All effect sizes are trivial or small, except the effect size for initial T_{SKIN} that can be classified as very large.

The reduction in T_{SKIN} was followed by a gradual increase that was observed until this temperature has reached a plateau; after that, T_{SKIN} remained elevated (around 33°C) until the rats had fatigued. The lower initial T_{SKIN} values observed in rats kept on the grid persisted until the 9th min of exercise. From then on, including at fatigue, no differences between trials were observed (Table 1).

Because the lack of bedding influenced T_{SKIN} , particularly at the beginning of exercise, we decided to maintain the control rats on the grid in the subsequent experiments.

Experiments after 24-h RSD or the control procedure

The analyses of time to fatigue in rats subjected to 24-h RSD or the control procedure indicated that the main effect of treatment did not reach statistical significance ($F = 2.76$; $p = 0.106$; Figure 2);

Figure 2. Time to fatigue in rats subjected to 24-h rapid eye movement sleep deprivation (RSD) or the control procedure; physical exercise was performed at two ambient temperatures: 24°C or 31°C (panel A). The data are expressed as means ± SEM and were analyzed using two-way ANOVAs, followed by Tukey's *post hoc* test. # denotes a significant ($p < 0.05$) main effect of ambient temperature. Panel B shows a small reduction in time to fatigue caused by RSD, irrespective of the ambient temperature. Panel C shows a large reduction in time to fatigue caused by the warm environment, irrespective of whether rats were or were not subjected to RSD.

indeed, RSD produced only a small magnitude effect in physical performance ($ES = 0.47$). In contrast, a significant main effect of ambient temperature was observed ($F = 15.39$; $p < 0.001$), with rats running 23% less at 31°C than at 24°C; the warmth-induced degradation of aerobic performance was classified as large effect size ($ES = 1.29$). Of note, no interaction was observed between these two factors ($F = 0.33$; $p = 0.570$), indicating that the effects of 24-h RSD on time to fatigue did not depend on the ambient temperature and vice-versa.

Treadmill running induced the expected increases in T_{CORE} and T_{SKIN} in all experimental trials. The increases in T_{CORE} and T_{SKIN} were more accentuated at 31°C than at 24°C; also, the increase in T_{SKIN} was observed after a short-latency period at 24°C but not at 31°C (Figure 3). We then performed additional analyses to better understand the influence of RSD and ambient temperature in T_{CORE} and T_{SKIN} at specific time-points (i.e., the beginning of exercise or at fatigue), as well as in the exercise-induced changes in these body temperatures. As detailed below, the exercise-induced changes in T_{CORE} were influenced by both the 24-h RSD and the warm environment, whereas the changes in T_{SKIN} were mainly influenced by the environment.

24-h RSD increased the rats' T_{CORE} at exercise initiation ($F = 11.65$; $p = 0.002$), but did not change this temperature at fatigue ($F = 0.28$; $p = 0.603$; Figure 4A,B), thus indicating the existence of a reduced exercise-induced change in T_{CORE} in sleep-deprived rats ($F = 6.29$; $p = 0.017$; Figure 4D,E). The RSD-induced effects in initial T_{CORE} (a 0.37°C increase) and in the T_{CORE} change (a 0.48°C reduction) were classified as moderate and small effect sizes, respectively ($ES = 1.15$ and 0.56). These sleep-deprived rats

Figure 3. Changes in colonic temperature (panels A and B) and tail-skin temperature (panels C and D) induced by physical exercise at 24°C or 31°C in control rats (white circles) or rats subjected to 24-h rapid eye movement sleep deprivation (RSD, black circles). The temperature data are shown until the exercise time point when the first rat stopped running, whereas the scatter plots with bi-directional error bars indicate the temperatures measured at fatigue. The data are expressed as means $\pm$ SEM and were analyzed using three-way ANOVAs. These analyses yielded the following significant results, which are similar for both panels: treatment effect ($p < 0.001$) and interaction between the ambient temperature $\times$ time ($p < 0.001$). No other significant interactions were observed.

did not present altered cutaneous heat loss, as indicated by the lack of a treatment effect in T_{SKIN} at exercise initiation ($F = 0.08$; $p = 0.775$) and at fatigue ($F = 0.03$; $p = 0.876$; [Figure 4G,H](#)). However, 24-h RSD produced a moderate increase in the threshold T_{CORE} for cutaneous heat loss ($F = 4.55$; $p = 0.041$; $ES = 0.68$; [Figure 4J,K](#)).

The warm environment did not influence T_{CORE} ($F = 0.19$; $p = 0.666$) but influenced T_{SKIN} ($F = 7.21$; $p = 0.011$) at exercise initiation. This result (i.e., a -1.49°C reduction in T_{SKIN} at 31°C ; large effect) is unexpected because both the exercise and heat exposure were initiated simultaneously; therefore, the exposure was not sufficiently long to change thermoregulatory parameters. As expected, the warm environment exaggerated the exercise-induced increases in T_{CORE} (Figure 4C) and T_{SKIN} , with both values being higher at fatigue during experiments performed at 31°C than at 24°C ($F = 39.61$ and $p < 0.001$ for T_{CORE} ; $F = 146.09$ and $p < 0.001$ for T_{SKIN} ; Figure

Figure 4. The colonic temperature at fatigue (panel A), exercise-induced changes in colonic temperature (panel D), the tail-skin temperature at fatigue (panel G), and colonic temperature threshold for cutaneous heat loss (panel J) in rats subjected to 24-h rapid eye movement sleep deprivation (RSD) or the control procedure; physical exercise was performed at two ambient temperatures: 24°C or 31°C. The data are expressed as means $\pm$ SEM and were analyzed using two-way ANOVAs, followed by Tukey's *post hoc* test. + denotes a significant ($p < 0.05$) main effect of experimental group. # denotes a significant ($p < 0.05$) main effect of ambient temperature. The panels B, E, H, and K show the effect sizes of the changes induced by RSD, irrespective of the ambient temperature. The panels C, F, I, and L show the effect sizes of changes induced by the warm environment, irrespective of whether rats were or were not subjected to RSD.

4F,I). These heat-induced greater body temperatures at fatigue were classified as very large effect sizes ($ES > 2.10$). The warm environment also induced a moderate reduction in the threshold T_{CORE} for cutaneous heat loss ($F = 4.15$; $p = 0.050$; $ES = 0.65$; Figure 4L).

Interestingly, as previously reported for physical performance, no interactions were observed between 24-h RSD and ambient temperature in any of the thermoregulatory responses determined in running rats, with p -values higher than 0.230 for each of the six responses evaluated (i.e., T_{CORE} at exercise initiation and fatigue, exercise-induced changes in T_{CORE} , T_{SKIN} at exercise initiation and fatigue, and the threshold for cutaneous heat loss).

Experiments after 96-h RSD or control procedure

The results observed following 96-h RSD were quite similar to those observed following 24-h RSD. The time to fatigue analysis indicated that 96-h RSD only produced a small ($ES = 0.58$), not statistically significant, reduction in physical performance ($F = 2.95$; $p = 0.096$; Figure 5). In contrast, the main effect of ambient temperature was observed, with rats running for shorter periods at 31°C than at 24°C ($F = 6.25$; $p = 0.018$); the average 6-min reduction in time to fatigue induced by the warm environment was classified as moderate effect size ($ES = 0.82$). No interaction was

Figure 5. Time to fatigue in rats subjected to 96-h rapid eye movement sleep deprivation (RSD) or the control procedure; physical exercise was performed at two ambient temperatures: 24°C or 31°C (panel A). The data are expressed as means $\pm$ SEM and were analyzed using two-way ANOVAs, followed by Tukey's *post hoc* test. # denotes a significant ($p < 0.05$) main effect of ambient temperature. Panel B shows a small reduction in time to fatigue caused by RSD, irrespective of the ambient temperature. Panel C shows a large reduction in time to fatigue caused by the warm environment, irrespective of whether rats were or were not subjected to RSD.

observed between these two factors ($F = 0.21$; $p = 0.652$), indicating that the effects of 96-h RSD on time to fatigue did not depend on the ambient temperature and vice-versa.

As observed for the experiments after 24-h RSD, treadmill running induced the expected increases in T_{CORE} and T_{SKIN} in all trials performed after 96-h RSD, with these changes being more accentuated at 31°C than at 24°C (Figure 6). Although a significant ambient temperature $\times$ treatment interaction was reported for T_{SKIN} ($F = 4.01$, $p = 0.014$), the *post hoc* analysis could not identify why the effect of ambient temperature on T_{SKIN} would be dependent on the treatment evaluated.

96-h RSD increased the rat's T_{CORE} at exercise initiation ($F = 5.631$; $p = 0.024$) but did not change this temperature at fatigue ($F = 0.624$; $p = 0.435$; Figure 7A,B), thus indicating the existence of a reduced exercise-induced change in T_{CORE} in sleep-deprived rats ($F = 5.568$; $p = 0.025$; Figure

Figure 6. Changes in colonic temperature (panels A and B) and tail-skin temperature (panels C and D) induced by physical exercise at 24°C and 31°C in control rats (white circles) or rats subjected to 96-h rapid eye movement sleep deprivation (RSD, black circles). The temperature data are shown until the exercise time point when the first rat stopped running, whereas the scatter plots with bi-directional error bars indicate the temperatures measured at fatigue. The data are expressed as means $\pm$ SEM and were analyzed using three-way ANOVAs. These analyses yielded the following significant results, which are similar for both panels: treatment effect ($p < 0.001$) and interaction between the ambient temperature $\times$ time ($p < 0.001$). A significant interaction between ambient temperature $\times$ treatment ($p = 0.014$) was only observed for tail-skin temperature. No other significant interactions were observed.

Figure 7. The colonic temperature at fatigue (panel A), exercise-induced changes in colonic temperature (panel D), the tail-skin temperature at fatigue (panel G), and colonic temperature threshold for cutaneous heat loss (panel J) in rats subjected to 96-h rapid eye movement sleep deprivation (RSD) or the control procedure; physical exercise was performed at two ambient temperatures: 24°C or 31°C. The data are expressed as means $\pm$ SEM and were analyzed using two-way ANOVAs, followed by Tukey's *post hoc* test. + denotes a significant ($p < 0.05$) main effect of experimental group. # denotes a significant ($p < 0.05$) main effect of ambient temperature. The panels B, E, H, and K show the effect sizes of changes induced by RSD, irrespective of the ambient temperature. The panels C, F, I, and L show the effect sizes of changes induced by the warm environment, irrespective of whether rats were or were not subjected to RSD.

7D,E). The RSD-induced effects in initial temperature (a 0.34°C increase) and in the T_{CORE} change (a 0.49°C reduction) were classified as moderate and small magnitude effect sizes ($ES = 0.78$ and 0.56 , respectively). Sleep-deprived rats presented lower T_{SKIN} at exercise initiation ($F = 21.639$; $p < 0.001$), a large effect mediated by 96-h RSD ($ES = 1.34$) that contrasts with results following 24-h RSD. Additionally, 96-h RSD did not influence the T_{SKIN} value at fatigue ($F = 0.607$; $p = 0.442$; Figure 7G,H) but produced a large increase in the threshold T_{CORE} for cutaneous heat loss ($F = 18.839$; $p < 0.001$; $ES = 1.45$; Figure 7J,K).

As expected, the warm environment exaggerated the exercise-induced increases in T_{CORE} (Figure 7C) and T_{SKIN} , with both values being higher at fatigue during experiments at 31°C than at 24°C ($F = 65.490$ and $p < 0.001$ for T_{CORE} ; $F = 41.661$ and $p < 0.001$ for T_{SKIN} ; Figure 7F,I). These heat-induced greater body temperatures at fatigue were classified as very large effect sizes ($ES > 2.20$). No significant effects mediated by the warm environment were observed in the T_{CORE} ($F = 0.008$; $p = 0.931$) and T_{SKIN} ($F = 0.587$; $p = 0.449$) at exercise initiation and in the threshold T_{CORE} for cutaneous heat loss ($F = 0.347$; $p = 0.560$; Figure 7L).

Again, no interactions were observed between 96-h RSD and ambient temperature in the thermoregulatory responses determined in running rats, with p -values higher than 0.210 for each of the six responses evaluated.

Discussion

This study investigated the effects of RSD, environmental heat stress, and the interaction between them on aerobic performance and exercise-induced changes in thermoregulatory responses. More specifically, this investigation was performed in rats subjected to two different durations of RSD (i.e., 24 h and 96 h). Our findings revealed that 24-h RSD produced a small magnitude effect on aerobic performance and modified thermoregulatory responses; a greater T_{CORE} was observed at exercise initiation but not at fatigue, thereby indicating a lower running-induced increase in T_{CORE} . Cutaneous heat loss through tail vessels was barely affected in sleep-deprived rats. Quite similar

effects on physical performance and thermoregulatory responses were detected following the more prolonged RSD protocol (i.e., 96 h). As expected, environmental heat stress increased T_{CORE} and T_{SKIN} values observed at fatigue and markedly reduced aerobic performance. More relevant, the performance and thermoregulatory effects mediated by RSD and environmental heat stress were, in all cases, independent from each other.

In the present study, small decreases in performance were reported in 24-h and 96-h sleep-deprived rats. This is a novel finding, as no previous studies have addressed the association between aerobic performance and RSD in laboratory animals. Several studies indicate that total sleep deprivation reduces performance in humans [10–12]. For instance, Martin [10] observed an 11% reduction in time to exhaustion during prolonged treadmill walking (80% $\text{VO}_{2\text{max}}$) following a 36-h sleep deprivation period, while Martin & Cheng [11] observed a 20% reduced time to exhaustion during constant-intensity walking protocol following 50 h of sleep deprivation. Using a more ecologically valid protocol to assess athletes' performance, Oliver et al. [12] observed a reduction in the distance covered during 30 min of self-paced treadmill running following one night of sleep deprivation. These studies suggest that the degraded aerobic performance caused by sleep deprivation might be attributed to changes in the brain, as limited effects were observed in cardiorespiratory or thermoregulatory functions [12] and the peripheral sympathetic nervous system [11]. The observation that subjects ran less distance after sleep deprivation than after a typical night but with a similar rating of perceived exertion in both conditions also supports the determinant role of central mechanisms [12].

Evidence indicates that RSD influences the production and release of neurotransmitters such as dopamine, noradrenaline, and acetylcholine [2,8,9,47]. Since these neurotransmitter systems' activity has been implicated in the modulation of aerobic performance [42,48,49], we hypothesized that RSD-induced changes in neurotransmitter levels would impair performance. In fact, RSD reduces dopamine levels without affecting serotonergic transmission in the striatum [9]. Considering that mice selectively bred for high

rates of voluntary exercise on running wheels have higher basal levels of DA in the caudate-putamen (i.e., dorsal striatum) [50,51], it is suggestive that reduced DA in the striatum is one of the central factors underlying decreased performance caused by RSD.

Regarding the thermoregulatory responses, RSD rats presented greater T_{CORE} at exercise initiation, a finding that agrees with other studies, in which short-term REM/total sleep deprivation increased the T_{CORE} of the animals [23–25]. Jaiswal et al. [24] observed marked hyperthermia ($\sim 1.2^{\circ}\text{C}$ rise) already on the 1st day of RSD; this hyperthermia was then gradually reversed with rats presenting normal T_{CORE} levels within 6–8 days of RSD. Interestingly, RSD was reported to increase noradrenaline both in the blood [20] and in the brain [48], and the RSD-induced hyperthermic response was blocked after systemic administration of prazosin, an α -1 adrenoceptor antagonist. Thus, according to Jaiswal et al. [24], the increased T_{CORE} in sleep-deprived rats results from augmented sympathetic outflow that induces peripheral vasoconstriction and increases metabolism.

The higher initial T_{CORE} in our sleep deprived-rats does not agree with findings in humans. Total sleep deprivation has induced either decreased [12,52] or unaltered baseline T_{CORE} [14] in humans, whereas REM sleep deprivation produced hypothermia in the first recovery night after deprivation [53]. The discrepancy in the thermoregulatory response between the two species (i.e., humans and rats) may result from the fact that sleep deprivation protocols are attained voluntarily in humans (which are aware of potential adverse effects) but involuntarily in rats. Thus, sleep deprivation protocols are likely more stressful in laboratory rodents than in humans. Elevated T_{CORE} is a common feature that has been reported in rats subjected to several experimental models for inducing psychological stress, such as the cage-switch stress [54], handling [55], and expectation for exercise initiation after being placed on the treadmill belt [56].

Exercise fatigue has been accepted as a brain-mediated process that depends on multiple afferent signals [57]. This afferent (sensory) feedback includes high T_{CORE} levels, a limiting factor for

aerobic performance [26,27,58,59]. In particular, higher T_{CORE} values at exercise initiation have been associated with a lower aerobic performance in rats [26,60,61], although this is not a universal finding [56]. In the present study, despite the fact that RSD rats presented greater T_{CORE} at exercise initiation and a higher heat loss threshold than control rats, no differences in T_{CORE} were observed at fatigue. Moreover, these findings mean that sleep deprived-rats presented lower exercise-induced changes in T_{CORE} , likely resulting from augmented heat loss or reduced metabolic rate during treadmill running.

The hypothesis of augmented heat loss through tail vessels seems unlikely, however. As indicated by T_{SKIN} values, cutaneous heat loss was barely affected by 24-h RSD; a higher T_{CORE} threshold for cutaneous heat loss was observed in sleep-deprived rats, even though this effect did not lead to greater T_{CORE} values at fatigue. The more prolonged RSD (i.e., 96 h) reduced T_{SKIN} at exercise initiation, but this response was no longer observed as rats were exercised. Because tail vasodilation is the main pathway by which rats dissipate body heat while running [44,62,63], alternative and maybe “underestimated” pathways for dissipating heat (e.g., evaporative breathing) may help explain the lower exercise-induced increase in T_{CORE} in sleep-deprived rats. Interestingly, a recent study failed to report changes in T_{CORE} during exercise, despite an apparent attenuation in the T_{SKIN} increase induced by caudal artery denervation [43].

To date, no study has investigated whether oxygen consumption (VO_2) is decreased in sleep-deprived rats subjected to treadmill running. However, the following two observations suggest that a lower VO_2 in sleep-deprived rats is very unlikely. First, men after 30 h without sleep presented slightly increased VO_2 while running at 60% of their $\text{VO}_{2\text{MAX}}$ [12]. Second, a reduced VO_2 during treadmill running would mean a decreased oxygen cost of locomotion, usually associated with increased performance, as recently observed in aerobically trained rats [64].

As expected [28,29,32], the aerobic performance was greatly impaired in the warm compared with the temperate environment, regardless of whether

the rats were subjected or not to sleep deprivation. The rats presented exaggerated body hyperthermia at 31°C relative to 24°C, as evidenced by higher T_{CORE} and T_{SKIN} at fatigue. The reduced performance under warm conditions has been attributed to greater hyperthermia [65], greater cardiovascular strain [66], and altered thermal perception [67]. Indeed, several psychophysiological mechanisms play a role in the association between hyperthermia and fatigue, including higher perceived exertion, reduced drive for skeletal muscle contraction, reduced cerebral blood flow, and endotoxemia [65].

Sleep quality has been listed as an intrinsic modulator that changes thermoregulation and the risk of developing heat stroke [68], a severe heat-related disorder [69]. In the present study, RSD did not exaggerate the exercise-induced increases in T_{CORE} , regardless of the environmental conditions. Notably, the magnitude of hyperthermia is positively associated with intestinal permeability during exercise [70]. Augmented intestinal permeability allows bacterial translocation [71], which may trigger a systemic inflammatory response that, under certain conditions, favors the occurrence of heatstroke [72]. Although we did not measure intestinal permeability and inflammatory response, any eventual impairment in gastrointestinal function induced by sleep deprivation would likely not result from altered thermoregulatory control, at least in the experimental conditions evaluated herein.

The changes in aerobic performance and thermoregulatory responses mediated by RSD and heat exposure were always independent of each other. For example, the reduced time to fatigue observed in sleep-deprived rats was not exaggerated when rats were exercised at 31°C. These findings suggest that the two experimental manipulations produce their effects acting by independent mechanisms. This hypothesis should be addressed in future analyses regarding the neuronal activation or neurotransmitter release in brain areas modulating thermoregulation and performance. Another hypothesis to explain the lack of interactions is that the exercise under environmental heat stress might induce a marked thermoregulatory strain that would override any impairment caused by RSD. This hypothesis is supported by the

observation that esophageal temperature was 0.3°C higher in individuals after 33 h of wakefulness; nevertheless, this difference was no longer observed during a subsequent exercise at 35°C [16].

We conclude that RSD and heat stress induced, respectively, small and large changes in the aerobic performance of running rats, thus indicating that degraded performance was more evident in the warm environment than after RSD. Besides, both experimental manipulations influenced thermoregulation in rats, even though RSD more clearly influenced thermoregulation in non-exercise conditions than during exercise. The warm environment, as expected, exaggerated the exercise-induced hyperthermia. Finally, the present data suggest that RSD and environmental heat stress modulate performance and thermoregulation by acting through independent mechanisms.

Disclosure statement

The authors have no potential conflicts of interest to disclose.

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