Proceeding of 4th International Conference on Research and Development (ICORAD)
Vol. 3 No. 2 (2024)
Page : 183-187
ISSN:2828-4925
DOI: 10.47841/icorad.v3i2.255

Circadian Analysis of Behavior Using Animal Activity Meter and Infrared Thermography in Rats

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Received: December,2, 2024 | Revised: December,18, 2024 | Accepted: December,20, 2024

Abstract. Rats are nocturnal animals that display heightened activity at night and variations in body temperature. Traditionally, behavioral studies have depended on observational methods; however, technological advancements, such as animal activity meters and infrared thermography, now provide more precise and non-invasive evaluations of both behavior and surface temperature measurement. These instruments possess significant potential for enhancing the precision and profundity of behavioral and physiological research. This study examined the exploratory behavior, locomotor activity, and surface temperature of rats during light and dark phases. Ten male Sprague-Dawley rats were kept on an artificial 12-hour dark/12-light cycle. The behavior and body surface temperature were measured twice daily, both during the dark cycle (6 PM) and at light cycle (6 AM). Their activity and behavior, including distance traveled, resting time, ambulatory time, and stereotypic time, were measured using the Opto Varimex-Auto Track system. The surface body temperature was measured at four regions (nose, mouth, testis, and abdomen) via an infrared thermal camera. The ambulatory time and distance traveled were statistically significantly higher during the dark cycle compared to during the light cycle. In contrast, the stereotypic time was statistically significantly higher during the light cycle compared to during the dark cycle. Despite the different intensity of activity between photoperiods, there was no significant difference in surface temperature between the dark and cycle. It is concluded that the behavior change in rats during their circadian rhythm can be measured using an animal activity meter.

Keywords: Circadian; Behaviour; Nocturnal; Rats; Temperature

INTRODUCTION

Circadian rhythms are 24-hour biological cycles that control any behavior or activity of a mammal, such as body temperature and hormonal secretion (W. Liu & Xuan, 2024). In the case of nocturnal animals such as rats (*Rattus norvegicus*), circadian rhythms are important since they allow for shifts in behavior and physiological processes to accommodate the ambient light conditions. In nature, bright light indicates a potential existential threat to them and elevates the risk of predation (Pellman et al., 2015). Rats typically avoid bright areas and favor darker environments. There are clear links between the number of activities within a 24-hour period, and changes in temperature. As body temperature rises at night, so does the propensity to engage in more active behaviors (A. C. Liu, Lewis, & Kay, 2007). Understanding such behaviours and physiological rhythms is important for many areas in biomedicine, such as neuroscience, pharmacology or toxicology among other fields.

Historically, behavioral observations in animal studies were conducted manually through laborious and subjective methods. While these methods have produced several findings in circadian behaviors, they are constrained by observer bias and limited observation periods. The introduction of digital instruments such as animal activity

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monitors and infrared thermography has facilitated more accurate, objective, and continuous evaluations of animal behavior and body temperature (Jourdan, Ardid, & Eschalier, 2001). Animal activity meters provide precise tracking of movement patterns, including distance traveled, walking activity, resting duration, and repetitive action over long periods. This offers a thorough and extensive view of an animal's circadian activity. Moreover, infrared thermography, a non-invasive technique for measuring surface temperature, facilitates the observation of subtle physiological alterations that accompany behavioral changes (Travain & Valsecchi, 2021). This integration has generated novel opportunities for research in circadian rhythm, improving our comprehension of the correlation between behavioral activity and thermoregulation in rats.

This study examined the relationship between behavioral circadian rhythm and surface body temperature in male Sprague-Dawley rats using advanced digital technological tools. We hypothesized that several behavior parameters, including distance traveled, ambulatory time, and stereotypic behaviors, would be markedly elevated during the dark phase, indicative of the natural nocturnal activity of rats. Furthermore, we aimed to examine if fluctuations in body surface temperature would correlate with behavioral changes between light and dark periods. The findings will enhance our knowledge of the influence of circadian rhythms on rats' exploratory and locomotor activities, therefore offering deeper insight into the physiological and behavioral mechanisms that govern circadian patterns.

METHOD

Experimental Design

This study used ten adult male Sprague Dawley rats housed at the university's laboratory animal facility. A specific room in the main campus building was utilized for recording behavior and conducting infrared thermography under controlled conditions. The animals were acclimatized to the new environment one week before the analysis. The rats were kept on an artificial 12-hour dark cycle (06:00 PM–06:00 AM) and 12-light cycle (06:00 AM–06:00 PM), with ad libitum access to water and feed. Data collection commenced everyday at 09:00 PM during dark period and at 09:00 AM during light period over a span of five consecutive days.

Behavioral recordings were performed with an Opto-Varimex-5 Auto Track System (Columbus Instrument-USA) for five minutes per session (Figure 1a), repeated three times for each rat. Recorded behavioral parameters included distance traveled, resting time, stereotypic activity (repetitive movements confined within box size limits), and ambulatory activity (movements exceeding these constraints). Additionally, surface temperatures across four regions (Figure 1c).-testes, abdomen, nose, and mouth-were measured via Flir One Pro infrared thermal camera (FLIR Systems Inc-USA).

Data Analysis

The animal's behaviors (distance traveled, resting time, stereotypic time, and ambulatory time) and surface temperature of testes, abdomen, mouth, and nose) were analyzed between light and dark cycles using GraphPad Prism 8.0 (GraphPad Software, LLC). Statistical differences were calculated by the Wilcoxon test with $P<0.05$ considered significant. The correlation between animal activities and surface temperature was calculated using the Spearman correlation test. The strength of correlation was determined

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by the range of absolute correlation *(r)*, very strong (0.8−1.0), strong (0.6−0.79), moderate (0.4−0.59), weak (0.2−0.39), and very weak (0−0.19).

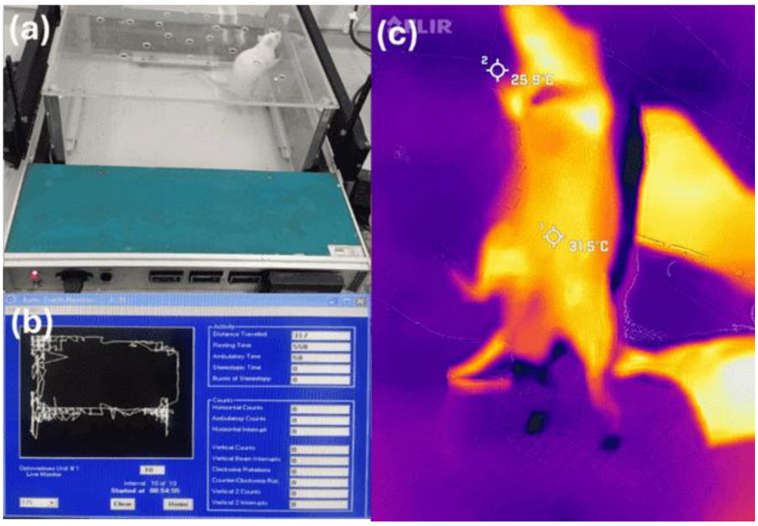

Figure 1 (a) Recording of rat's behavior using an animal activity meter, (b) results of the animal behavior measurements, (c) infrared thermography image of the rat

RESULTS AND DISCUSSION

Rats are intrinsically nocturnal animals that are biologically programmed to be more active during the night. Rats have markedly increased locomotor activity during the night time compared to the day time. Our finding demonstrated that rats exhibited a statistically significant increase ($p<0.05$) in distance traveled (Figure 2a) and ambulatory time (Figure 2b). Enhanced running activity during the nocturnal phase was also seen in rats provided with access to running wheels (Boakes & Wu, 2021). In nature, nocturnal animals utilize the dark phase to cultivate essential survival skills, including foraging and hiding from predators. The circadian rhythm represents the adaptation of animals to diurnal variation and is regulated by melatonin secretion from pineal gland, which is maximally secreted during the dark period. The melatonin secretion progressively increases at the cessation of light during the photoperiod (Urbanski, 2000). The magnitude of various exploratory behaviors was directly proportional to the plasma melatonin concentration (Molcan et al., 2019). Conversely, during the light phase, rats demonstrate diminished activity levels and prolonged resting period (Figure 2c). The physiological mechanisms driving this behavior involve hormonal variations; specifically, corticosterone levels peak just before the onset of darkness and decline throughout the dark phase (Dauchy et al., 2010). Disruption of the light cycle, such as during a solar eclipse, induces the appearance of nocturnal behavior within the light cycle (Tarigan, Achmadi, Bustamam, & Santoso, 2023).

Stereotypies are a type of psychotic behavior characterized by immobility and repetitive, purposeless motions, including body rocking and repetitive hopping (Mason, 1991). Stereotypies frequently emerge from stress and dissatisfaction induced by constrained captive settings, sudden environmental alterations in wild and domesticated animals, and the use of psychoactive medications (Carlstead, Brown, & Seidensticker, 1993). Stereotypies are considered pathological indicators of suffering and inadequate welfare in caged animals (Broom, 1983). Our study suggests an increase in stereotypic behavior during light periods (Figure 2d) may indicate elevated anxiety or stress levels

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when they are unable to engage in normal exploratory activities. Stereotypies may arise as coping mechanisms when animals are facing environmental challenges. The inability to manage a stressor adversely affects animals, leading to cardiovascular pathology, ulcer formation, and infectious infections (Koolhaas et al., 1999).

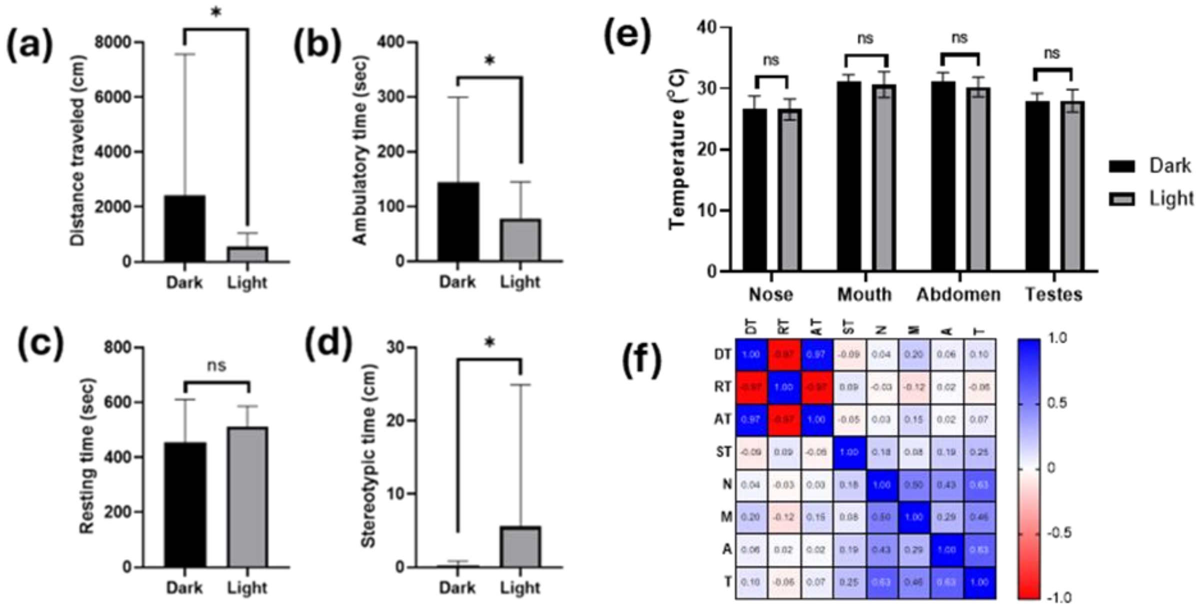

Figure 2 (a) Distance travelled, (b) ambulatory time, (c) resting time, (d) stereotypic time, and (e) surface temperature of several regions of rats during dark and light cycle, (f) Pearson correlation matrix between behavior and surface temperature. * p < 0.05, ns: not significant, DT: distance traveled, RT: resting time, AT: ambulatory time, ST: Stereotypic time, N: nose, M: mouth, A: abdomen, T: testes, very strong correlation (r: 0.8–1.0), strong correlation (0.6–0.79), moderate correlation (0.4–0.59), weak correlation (0.2–0.39), and very weak correlation (0–0.19).

As nocturnal animals, rats exhibit heightened activity during the dark phase and often repose during the day phase (Figure 2a–d). Nonetheless, despite these behavioral variations, their surface temperatures stay stable during both light and dark periods (Figure 2e) and exhibit a very weak correlation (Figure 2f) with all observed behaviors (distance traveled, resting time, ambulatory time, and stereotypic time). Rats maintain homeostasis through thermoregulation mechanisms involving behavioral adaptations (e.g., seeking cooler or warmer areas) and physiological responses (e.g., vasodilation or vasoconstriction). The absence of significant temperature variation may indicate that rats effectively manage their body temperature regardless of activity levels or environmental conditions.

CONCLUSION

As nocturnal animals, rats showed increased exploratory behavior during dark periods and increased stereotypic behavior during light periods without any difference in their surface temperature that explained their thermoregulatory ability.

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