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Original article

Short-term sleep deprivation with exposure to nocturnal light alters mitochondrial bioenergetics in Drosophila

Nathane Rosa Rodrigues[a,b], Giulianna Echeverria Macedo[a], Illana Kemmerich Martins[a], Karen Kich Gomes[a], Nélson Rodrigues de Carvalho[a], Thaís Posser[a], Jeferson Luis Franco[a,b,*]

[a] Oxidative Stress and Cell Signaling Research Group, Centro Interdisciplinar de Pesquisas em Biotecnologia – CIPBIOTEC, Universidade Federal do Pampa, Campus São Gabriel, RS, Brazil

[b] Departamento de Bioquímica e Biologia Molecular, CCNE, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil

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ABSTRACT

Many studies have shown the effects of sleep deprivation in several aspects of health and disease. However, little is known about how mitochondrial bioenergetics function is affected under this condition. To clarify this, we developed a simple model of short-term sleep deprivation, in which fruit-flies were submitted to a nocturnal light condition and then mitochondrial parameters were assessed by high resolution respirometry (HRR). Exposure of flies to constant light was able to alter sleep patterns, causing locomotor deficits, increasing ROS production and lipid peroxidation, affecting mitochondrial activity, antioxidant defense enzymes and caspase activity. HRR analysis showed that sleep deprivation affected mitochondrial bioenergetics capacity, decreasing respiration at oxidative phosphorylation (OXPHOS) and electron transport system (ETS). In addition, the expression of genes involved in the response to oxidative stress and apoptosis were increased. Thus, our results suggest a connection between sleep deprivation and oxidative stress, pointing to mitochondria as a possible target of this relationship.

1. Introduction

The hectic lifestyle of modern society causes several harmful consequences to health. Sleep deprivation and related-diseases are among them being 20% of the adult population affected by this condition [1]. Two processes are responsible for regulating sleep: (i) a circadian clock that regulates sleep time and (ii) a homeostatic mechanism that perceives and responds to lack of sleep [2,3]. The daily variations during rest and activity periods are among the most observable circadian changes [4] and can be influenced by environmental factors, such as temperature changes, light cycles and food availability, which leads to the trapping of the circadian cycle [5].

Sleep is a biological event observable in most species, however, its functions are not clear [6]. However, it is known that sleep plays a critical role in learning and consolidating newly acquired memories [7], as well as in survival since studies in rats and flies have shown that long-term sleep deprivation results in death [3,8,9]. In addition, increasing peaces of evidence suggests the physiological role of sleep in the metabolic process, response to stress and inflammation, energy recovery among others [10,11].

Sleep deprivation has been related to elevated levels of reactive oxygen species (ROS) [12], however this relationship is not well understood. ROS are by-products of the normal metabolism of mitochondria, formed in part during the electron transfer process that occurs along the electron transport chain (ETC), in order to produce energy in the form of adenosine triphosphate (ATP) during oxidative phosphorylation (OXPHOS) [13]. In addition to the production of ATP, mitochondria exert essential functions for the cell, such as energy metabolism, production and elimination of ROS, calcium homeostasis, survival and cell death. Thus, mitochondrial dysfunctions such as decreased oxidative capacity and antioxidant defense, reduced OXPHOS and decreased ATP production are related to metabolic disorders [14]. A study in sleep-deprived mice demonstrated a decrease in mitochondrial complexes activity and provides evidence that mitochondrial dysfunction is involved in the regulation of sleep recovery [15].

In Drosophila and mammals, three gene categories have been shown to be consistently up-regulated during wakefulness and sleep deprivation, including genes involved in energy metabolism, synaptic potentiation, and cellular stress response [16]. The rhythmic expression of genes involved in metabolic pathways and resistance to stress was suggested from studies of microarrays in complete Drosophila genome [17]. Many antioxidants and enzymes that protect the cell from

* Corresponding author at: Oxidative Stress and Cell Signaling Research Group, Centro Interdisciplinar de Pesquisas em Biotecnologia – CIPBIOTEC, Universidade Federal do Pampa, Campus São Gabriel, São Gabriel, RS 97300-000, Brazil.
E-mail address: jefersonfranco@unipampa.edu.br (J.L. Franco).

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oxidative stress such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx) and peroxiredoxin (Prx) show oscillation in their expression or levels of activity according to the circadian rhythm, in the same way, levels of oxidative stress by products, such as those indicating DNA damage, protein damage or lipid peroxidation, also oscillate at different times of the day [18], suggesting that this rhythmicity in antioxidant defenses can protect the body from excessive levels of ROS and its resulting damage to biological macromolecules [19]. However, the phase, amplitude and levels of different antioxidants and/or pro-oxidants may vary depending on specific tissues and organisms [20]. For instance, in humans, some antioxidant enzymes have a morning peak, while melatonin and lipid peroxidation show evening peaks [18].

In the last decade, *Drosophila melanogaster* emerged as a powerful model to investigate sleep and its characteristics [21–24]. Drosophila has many similarities to mammalian sleep, such as prolonged periods of immobility associated with increased excitation thresholds, varying amounts of sleep required by age (young flies sleep longer), responses to chemical stimulants, gene modulation for sleep and wakefulness [25,26]. Furthermore, the two driving forces for sleep are both conserved in Drosophila: the interaction between intrinsic circadian rhythms and external environmental cues and a need for natural sleep regulated by sleep homeostasis [27]. Recently, fruit fly research that collaborated to decipher the regulation of circadian rhythm was awarded the Nobel Prize in Physiology or Medicine in 2017 to researchers Jeffrey C. Hall, Michael Rosbash and Michael W. Young. All these characteristics, together with the development of tools for observation and sleep analysis, make Drosophila an attractive and valid model for sleep studies.

Thus, this study aimed to investigate the relationship between changes in sleep patterns, mitochondrial functions and expression of oxidative pathways genes in the *Drosophila melanogaster* model, using a simple sleep deprivation protocol via exposure to nocturnal light as an environmental cue.

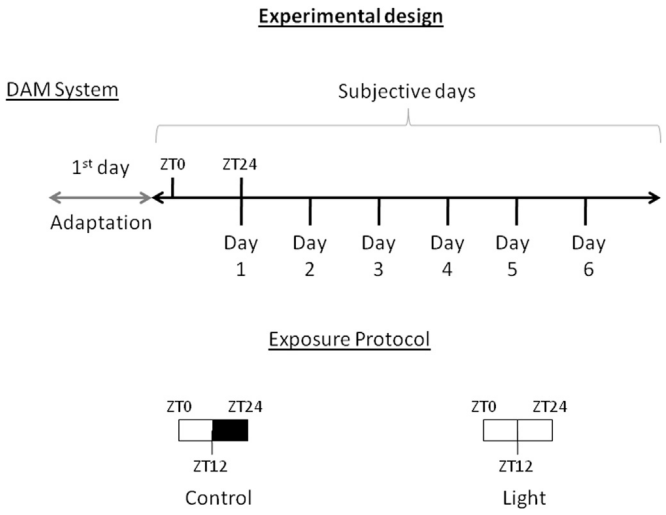
2. Methods

2.1. Fly stock and treatments

D. melanogaster (Oregon strain) was obtained from the National Species Stock Center, Bowling Green, OH, USA. The flies were maintained in incubators at 25 ± 1 °C, 12 h dark-light photoperiod and 60–70% relative humidity with free access to food as previously described [28]. For the experiments, female flies (1–4 days-old) were placed in tubes containing agar meal during 24 h under controlled conditions of light as follows control group (12 h light/12 h dark cycle), and Light group (12 h light/12 h light cycle), both at 25 ± 1 °C (Scheme 1). After treatments period, always finished at 12:00 p.m., the flies were submitted to behavioral tests, biochemical and molecular analysis.

2.2. Sleep analysis

For the sleep analysis and circadian rhythm measurements we used the Drosophila Activity Monitor (DAM) (DAMSystem 308-TriKinetics Inc, Waltham, MA) in which a single fly at a time is allocated in glass tubes (5 × 65 mm) containing agar medium (1.5%) at one end and arranged horizontally on specific monitors supporting up to 32 tubes. During the experiments, the monitors were maintained in a DigiTherm® CircKinetics™ incubator (Tritech Research Inc, Los Angeles, CA) set at 25 °C. As the fly passes through the monitor's infrared beams, its activity is registered. Thus, female flies with 1–4 days-old were maintained under treatments conditions for six days, the first day was not considered in the sleep analysis since at this period flies were allowed to adapt to the system's conditions. As stated, control group was exposed to 12:12 h light/dark cycle, and the Light group was exposed to constant light 12:12 h light/light (Scheme 1). Aiming to evaluate the sleep deprivation without circadian clock interferences, a separated experiment was performed in which flies were allocated into DAM's glass tubes containing agar medium and arranged horizontally on specific monitors. The monitors were then placed over the platform of an orbital shaker (Labnet Enduro™ Mini Mix™ 3D S0600) set at 20 rpm and the experiment was conducted at 12:12 h light/dark cycle (Shake group). For the sleep, circadian rhythm and activity analysis the first 24 h after adaptation period were considered. Sleep patterns were analyzed on pySolo program which converts the results from DAM to graphical activity data, sleep cycles, sleep quantity, and sleep fragmentation among others [29]. For sleep experiments, a total of sixty-four individual flies were used per group (n = 64). All experiments were performed in duplicates.



Scheme 1. Experimental design. *D. melanogaster* with 1–4 days old were maintained in the DAMSystem (Drosophila Activity Monitor) for 7 days. The first day was disregarded (phase of adaptation to the system). Under standard conditions (Control) the Zeitgeber (ZT) started at 7:00 a.m. with the lights ascending (ZT0) and hung up at 7:00 p.m. with the lights off (ZT12), each day of experiment was considered to complete ZT cycle (ZT0–24 − 7:00–6:59 h). For the Light group, the light remained constantly on throughout the experimental period (7 days). For the other experiments performed outside the DAM system, the control group flies were kept in a light / dark cycle of 12 h and the flies of the Light group were kept under constant light for a total period of 24 h, both were kept under temperature of 24 °C.

2.3. Locomotor activity

Locomotor activity was determined as negative geotaxis behavior (climbing ability) in individual flies [30] with some modifications. In brief, after the treatment is finished, a total number of 20 flies *per* group were anesthetized on ice and individually placed in vertical glass tubes (length 25 cm, diameter 1.5 cm) closed with cotton wool. After 30 min of recovery the flies were gently tapped to the bottom of the tube and the time taken by each fly to climb 6 cm in the glass column was recorded. The test was repeated 3 times with 20 s intervals for each fly. A total of twenty individual flies were used per group (n = 20). All experiments were performed in duplicates. The results were expressed as percentage of control.

2.4. Enzyme assays

For measurements of enzymes activity, twenty flies per group were homogenized in 20 mM HEPES buffer (pH 7.0). The homogenate was centrifuged at 20,000*g* for 30 min (4 °C) (Eppendorf 5427R, rotor FA-45-30-11). The supernatant was isolated and used for measuring the activity of antioxidant enzymes (superoxide dismutase, catalase,