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Aging induced endoplasmic reticulum stress alters sleep and sleep homeostasis

Marishka K. Brown [a], May T. Chan [a], John E. Zimmerman [a], Allan I. Pack [a,b], Nicholas E. Jackson [a], Nirinjini Naidoo [a,b,*]

[a] Center for Sleep and Circadian Neurobiology, University of Pennsylvania, Philadelphia, PA, USA
[b] Division of Sleep Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

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ABSTRACT

Alterations in the quality, quantity, and architecture of baseline and recovery sleep have been shown to occur during aging. Sleep deprivation induces endoplasmic reticular (ER) stress and upregulates a protective signaling pathway termed the unfolded protein response. The effectiveness of the adaptive unfolded protein response is diminished by age. Previously, we showed that endogenous chaperone levels altered recovery sleep in *Drosophila melanogaster*. We now report that acute administration of the chemical chaperone sodium 4-phenylbutyrate (PBA) reduces ER stress and ameliorates age-associated sleep changes in *Drosophila*. PBA consolidates both baseline and recovery sleep in aging flies. The behavioral modifications of PBA are linked to its suppression of ER stress. PBA decreased splicing of X-box binding protein 1 and upregulation of phosphorylated elongation initiation factor 2 α, in flies that were subjected to sleep deprivation. We also demonstrate that directly activating ER stress in young flies fragments baseline sleep and alters recovery sleep. Alleviating prolonged or sustained ER stress during aging contributes to sleep consolidation and improves recovery sleep or sleep debt discharge.

1. Introduction

Aging involves progressive deterioration of many physiological functions over time. Many age-associated diseases such as Alzheimer's disease, Parkinson's disease, and type II diabetes are partially characterized by accumulation and aggregation of misfolded proteins, indicating a decline in quality control and chaperoning systems (Naidoo, 2009a). One such quality control system, the endoplasmic reticulum (ER) stress response also known as the unfolded protein response (UPR), is responsible for maintaining protein homeostasis in the ER, the location for synthesis, processing, folding, and posttranslational modifications of all secretory and integral membrane proteins. Alterations in ER homeostasis disrupt proper folding and lead to accumulation of misfolded proteins, which are deleterious to cell survival. A variety of physiological conditions can provoke ER stress, including glucose or energy deprivation, redox changes, and alterations in calcium signaling. The UPR (Schroder and Kaufman, 2005; Zhang and Kaufman, 2006) has 3 distinct cellular responses: (1) upregulation of distinct molecular chaperones such as immunoglobulin binding protein and/or glucose-regulated protein 78(BiP/GRP78) through the inositol-requiring enzyme-1 (IRE1) pathway; (2) attenuation of protein translation mediated by the serine–threonine kinase PKR-like ER kinase (PERK), which phosphorylates the eukaryotic initiation factor 2α (eIF2α), subsequently reducing translation; and (3) degradation of misfolded proteins by ER-associated degradation (Harding et al., 1999). Excessive or extended ER stress leads to a maladaptive response and apoptosis, through activation of caspases and/or c-Jun-N-terminal kinase (JNK) signaling pathways (Szegezdi et al., 2006; Wu and Kaufman, 2006).

Prolonged wakefulness or sleep deprivation activates the UPR in mice (Naidoo et al., 2005) and the fruitfly *Drosophila melanogaster* (Naidoo et al., 2007; Shaw et al., 2000). Additionally, the UPR influences recovery sleep following sleep loss. Overexpression of BiP, also known as heat shock cognate 70 in *Drosophila,* results in increased recovery sleep when compared with sleep deprived wild-type controls (Naidoo et al., 2007). Further, animals that had reduced levels of functional BiP recovered less sleep after deprivation. These results are particularly pertinent in the context of baseline sleep and recovery sleep in the aged/elderly. Impairments in sleep architecture and sleep consolidation, including an increase in excessive daytime sleepiness (EDS), nighttime awakenings,

* Corresponding author at: Center for Sleep and Circadian Neurobiology, University of Pennsylvania Perelman School of Medicine, 2100 Translational Research Laboratories, 125 South 31st Street, Philadelphia, PA 19104-3403, USA. Tel.: +215 746 4811; fax: +215 746 4814.
E-mail address: naidoo@mail.med.upenn.edu (N. Naidoo).

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and reductions in recovery sleep, are well documented in aging populations (Mendelson and Bergmann, 2000; Pandi-Perumal et al., 2002; Wolkove et al., 2007). EDS is associated with significant negative health consequences including increased incidence of functional impairments (Lee et al., 2007) and mortality (Empana et al., 2009). EDS is also one of the most prevalent features of neurodegenerative diseases (Kato et al., 2012). Basal expression of BiP as well as other UPR components decreases with age (Naidoo, 2009b). Collectively, these results suggest that the amount of chaperone present influences the amount of sleep recovered after sleep loss (Naidoo et al., 2007).

In this study, we examined the role of ER stress in sleep and sleep homeostasis. First, we wanted to determine if supplementing basal levels of endogenous molecular chaperones with a chemical chaperone would alleviate ER stress and alter baseline and recovery sleep in aged flies. Secondly, we sought to ascertain whether inducing ER stress in young flies would confer an aged phenotype. Lastly, we examined the effect of the chemical chaperone on sleep behavior in a short-sleeping mutant. The chemical chaperone we chose is sodium 4-phenylbutyrate (PBA), which is a nonselective chaperone that binds to the exposed hydrophobic regions of misfolded proteins. It has been shown to stabilize protein conformation, improve the folding capacity of the ER, and facilitate the trafficking of mutant proteins (Ozcan et al., 2006). We wanted to establish whether acute administration of PBA would alter the UPR response and/or modify sleep behavior. We assessed sleep in aging populations of *Drosophila* and demonstrated consolidation of baseline sleep in aging flies by application of a clinically relevant dose of PBA. We also show that recovery sleep is altered in aged populations of flies and that PBA ameliorates some of these age-related sleep changes. We found that tunicamycin treatment, which induces ER stress, fragments baseline sleep, and alters recovery sleep, demonstrating a direct link between ER stress and sleep. We also illustrate that PBA treatment consolidates sleep in a short-sleeping mutant. These results demonstrate a correlation between the improvements in sleep by PBA application and attenuation of the IRE1 and PERK pathways of the UPR.

2. Methods

2.1. Fly stocks and maintenance

The *D. melanogaster* strain white Canton-Special (wCS10), a gift from Ronald Davis, Baylor College of Medicine (Houston, TX, USA), w^{1118ex} from Bloomington Stock Center (Bloomington, IN, USA) and sleepless (sss^{p1}) and its background control strain iso^{31}, a gift from Amita Sehgal and Kyunghee Koh (University of Pennsylvania and Thomas Jefferson University, Philadelphia, PA, USA) were used in these studies. All flies were maintained at room temperature on standard food that contained molasses or dextrose, cornmeal, and yeast on a 12 h: 12 h light: dark cycle. Flies were transferred onto new food every 2–3 days. *wCS10* maximum life span = 93 days, w^{1118ex} = 73 days, *ctrl sss*, isogenic wild-type strain, iso^{31} = 101 days, and sss^{p1} = 50 days. Flies were divided into age groups as follows: *wCS10* young (9–12 days) and aged (8 weeks). At 8 weeks >40% of these animals were still alive. For the w^{1118ex}, iso31, and sss^{p1} strains, flies were young (7 days) and aged (5–6 weeks).

2.2. Drug administration

PBA and tunicamycin were purchased from Calbiochem, EMD Chemicals Inc (Gibbstown, NJ, USA). The purity of PBA was 99.6%. PBA was diluted in deionized distilled water. 5 mM PBA was chosen as the preferred dose from a survival curve using 1 mM, 5 mM, and 10 mM. An acute treatment of PBA was shown to be more beneficial than a continuous dose on life span (Zhao et al., 2005). Tunicamycin was prepared in 95% ethanol for a stock solution of 1.19 mM. For both PBA and tunicamycin treatment, flies were placed into locomotor tubes containing the sucrose and/or agar media and drug (5 mM PBA or 12 μM tunicamycin) or vehicle (distilled water or 0.01% ethanol).

2.3. Circadian and behavioral sleep assays

Flies were collected one day after eclosion and housed in groups until behavior was recorded using video (see section 2.5). For all behavioral experiments, *D. melanogaster* virgin females were placed in individual locomotor activity tubes onto plates that hold 28 animals. Each locomotor activity tube contained a minimal media food mixture that consisted of 5% sucrose 1% agar media with or without 5 mM of PBA. Flies were allowed to acclimate to the tubes at least 24 hours before the recordings began. Baseline sleep was recorded for 2 days. Sleep was defined as a 5-minute bin without activity (Andretic and Shaw, 2005). Sleep and/or wake behavior was monitored throughout the study. We performed 15 baseline experiments of 28 flies each with young *wCS10* flies. For the aged *wCS10* flies, we performed 10 baseline experiments of 28 flies each. For the $w1118^{ex}$ strain, we performed 4 behavioral baseline experiments in the young flies and 4 experiments in the aged flies. The tunicamycin baseline experiments consisted of 4 experiments. The flies were treated with tunicamycin 24 hours before recording sleep behavior. We performed 5 experiments for the sss^{p1} mutants and their background controls iso^{31}.

2.4. Sleep deprivation (SD) and recovery

Baseline behavior was recorded for 2 days before SD. Flies were sleep deprived for 6 hours during the consolidated rest period from ZT 18 to ZT 24 (Naidoo et al., 2007). This task was accomplished manually by gently tapping the plates as necessary to keep the flies moving as determined by visual observation of fly behavior under red light conditions. We estimated that flies lost about 90%–100% of their sleep based on previously published studies where sleep loss during SD was measured using the same method (Naidoo et al., 2007). Time and aged matched control flies were maintained in the monitors without intervention until sacrificed.

Recovery sleep was recorded for 24 hours immediately following sleep deprivation beginning at ZT0. There were 12 recovery experiments performed for young *wCS10* flies and 8 experiments for the aged *wCS10* flies (n = 28/experiment). For w^{1118ex}, we performed 4 recovery experiments for the young and 4 experiments in the aged flies (n = 28/experiment).

The tunicamycin studies consisted of 4 experiments (n = 28/experiment). For these sets of experiments, flies were placed on tunicamycin 18 hours before SD. Each experiment consisted of 28 flies each recorded in a separate locomotor tube. Flies that died before the end of the experiments were not included in the behavioral analysis.

2.5. Video recording and analysis

Flies were recorded using the video system previously described (Zimmerman et al., 2008). Images were acquired at 5-second intervals using a Retiga 2000R camera (Qimaging, Surrey, British Columbia, Canada) and custom software written using MatLab (Mathworks, Natick, MA, USA). Infrared LED lamps (Lilin Corp., Arcadia, CA, USA) at a peak wavelength of 850 nm were used for camera illumination during the dark period. Video analysis: custom software written with Matlab and C computer languages was used to analyze the video images using subtraction analysis. Corresponding pixels from 2 temporally adjacent images are subtracted and each