Biochemical and Biophysical Research Communications xxx (2017) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Short-term exposure to dim light at night disrupts rhythmic behaviors and causes neurodegeneration in fly models of tauopathy and Alzheimer's disease

Mari Kim [b, 1], Manivannan Subramanian [a, 1], Yun-Ho Cho [a], Gye-Hyeong Kim [a], Eunil Lee [b], Joong-Jean Park [a, *]

[a] Department of Physiology, College of Medicine, Korea University, 73 Inchon-ro, Seongbuk-gu, Seoul 02841, Republic of Korea
[b] Department of Preventive Medicine, College of Medicine, Korea University, 73 Inchon-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

ARTICLE INFO

Article history:
Received 29 November 2017
Accepted 4 December 2017
Available online xxx

Keywords:
Alzheimer's disease (AD)
Circadian rhythm (CR)
Sleep
Dim light at night (dLAN)
Neurodegeneration
Drosophila melanogaster

ABSTRACT

The accumulation and aggregation of phosphorylated tau proteins in the brain are the hallmarks for the onset of Alzheimer's disease (AD). In addition, disruptions in circadian rhythms (CRs) with altered sleep-wake cycles, dysregulation of locomotion, and increased memory defects have been reported in patients with AD. *Drosophila* flies that have an overexpression of human tau protein in neurons exhibit most of the symptoms of human patients with AD, including locomotion defects and neurodegeneration. Using the fly model for tauopathy/AD, we investigated the effects of an exposure to dim light at night on AD symptoms. We used a light intensity of 10 lux, which is considered the lower limit of light pollution in many countries. After the tauopathy flies were exposed to the dim light at night for 3 days, the flies showed disrupted CRs, altered sleep-wake cycles due to increased pTau proteins and neurodegeneration, in the brains of the AD flies. The results indicate that the nighttime exposure of tauopathy/AD model *Drosophila* flies to dim light disrupted CR and sleep-wake behavior and promoted neurodegeneration.

1. Introduction

Alzheimer's disease (AD) is one of the most common forms of dementia all over the world. The onset of AD is associated with the accumulation of β-amyloid peptide (Aβ) and/or phosphorylated tau [1]. Tau stabilizes microtubules, which are involved in neuronal axon transport [2]. In patients with AD, hyperphosphorylated tau is segregated from microtubules, which results in the loss of microtubule integrity. In addition, hyperphosphorylated tau accumulates in patients with AD and forms intracellular neurofibrillary tangles (NFTs), which become neurotoxic and eventually cause neurodegeneration [3].

Rhythmic behaviors are important in normal physiological processes. Two common and interacting rhythmic behaviors are circadian rhythms (CRs) and sleep-wake behaviors. CRs are involved in the regulation of the sleep-wake behavior, and disruptions in CRs eventually result in changes in sleep. Normal sleep is required to restore neural plasticity and function [4] and clear toxic compounds that accumulate during the daytime [5]. Studies conducted using AD model mice have shown that the CRs of the mice are altered by the accumulation of Aβ and the formation of NFTs [6]. Sleep is profoundly affected in patients with AD showing altered sleep-wake behavior with reduced night sleep. Mouse models exhibiting AD phenotypes also show decreased sleep at night [6]. Thus, sleep disturbances are considered an indicator of the pathogenesis of AD [7]. Even in healthy individuals, decreased sleep enhances the accumulation of Aβ peptides and the formation of NFTs [8]. Thus, sleep and AD are interlinked, and any perturbations of one will affect the other [9].

The interference of CRs and/or sleep by light at night is referred to as light pollution, which has recently emerged as a major health problem in many developed countries, where any nighttime exposure to light that is over 10 lux in a residential area is prohibited. In modern industrialized societies, shift workers are continuously exposed to light at night, which disrupts their CRs and sleep-wake behaviors [10]. Shift workers have recently been reported to be more susceptible to cardiovascular disease, diabetes, obesity, cancer, and metabolic disorders [11,12]. Studies conducted

* Corresponding author.
E-mail address: parkjj@korea.ac.kr (J.-J. Park).
[1] Contributed equally on this work.

https://doi.org/10.1016/j.bbrc.2017.12.021
0006-291X/

on mice have also shown that disruptions of CRs by exposure to dim light (10 lux) at night leads to increased tumorigenesis with reduced lifespans [11]. Because CRs are regulated by the nervous system, any negative effects of CR disruptions on other systems should appear rapidly.

Drosophila melanogaster has been serving as a useful model system for investigating the genetic bases of CRs and sleep [13] and the pathogenesis of AD [14,15]. Previous studies on *Drosophila* have reported that exposure to moonlight at night alters CRs [16]. However, it remains unclear whether exposure to 10 lux of dLAN affects rhythmic behaviors and/or neurodegeneration in AD model organisms. In this study, we investigated the effects of short-term exposures to dim light on physiology in AD flies and found that AD flies were more vulnerable to the negative effects of the dim light exposure.

2. Materials and methods

2.1. Drosophila strains

The flies were cultured on cornmeal-based standard food under a 12:12-h light-dark (LD) cycle at 25 °C with 40–60% relative humidity [17]. *Elav-GAL4* (*Elav^{C155}-GAL4*) and *GMR-GAL4* fly stocks were obtained from Bloomington *Drosophila* Stock Center (Bloomington, IN, USA). Other stocks used were *UAS-hTau^{WT}*, *UAS-hTau^{RW}* [18], and *w^{1118}* (+/+) for the wild type.

2.2. Circadian rhythms and sleep

Freshly eclosed *Drosophila* males were collected and transferred into 3-mm-diameter glass tubes containing food at one end. These flies were monitored for 6 days using the *Drosophila* Activity Monitoring System (TriKinetics, Inc., Waltham, MA, USA) in an incubator with a 12:12-h LD cycle. For the first 3 days, the flies were maintained under a normal LD cycle, and then exposed 10 lux during the dark cycle for the next 3 days. Locomotor activity was recorded for 30-min intervals, and rhythmicity and free-running periods were calculated using a cosinor analysis. Sleep behaviors were analyzed during the 3rd day of each lighting pattern (LD3 or LL3) using pySolo software. Sleep deprivation was calculated by subtracting the sleep time recorded on the third day (LD3 and LL3) during the 10-lux light exposure from the sleep time recorded during 0 lux in each treatment group. Similarly, the amount of sleep recovered from ZT09 to ZT21 the day after the third day was calculated [19].

2.3. Lifespan assay

The flies were raised at 25 °C under 50% relative humidity and a 12:12-h LD cycle, and the food vials were replaced every 2–3 days [17]. Dead flies were counted every 2–3 days, and the survival curves were plotted using Prism software (v6.05; GraphPad Software).

2.4. Western blotting

Adult fly heads were homogenized in HEPES-EDTA lysis buffer and centrifuged at 13,000 rpm for 10 min. The supernatant was collected and loaded onto SDS-polyacrylamide gel and transferred to nitrocellulose membrane. The membrane was blocked with 5% skim milk and incubated overnight at 4 °C with anti-tau and anti-pTau antibodies (both 1:1000; Thermo Fisher Scientific) followed by incubation with secondary antibody (1:10,000; Cell Signaling Technology) and visualized with chemiluminescent detection. A mouse β-actin antibody (1:1000; Cell Signaling Technology) was used as a loading control.

2.5. Immunohistochemistry

Adult fly heads were fixed in 4% paraformaldehyde and washed with PBS. The brains were blocked with 5% normal goat serum in PBS containing 0.1% Triton-X 100 (PBST) and incubated with murine anti-tau and rabbit anti-pTau antibodies for 48 h at 4 °C. After washing with PBST, the samples were incubated with an anti-mouse secondary antibody conjugated with Alexa Fluor 488 and an anti-rabbit secondary antibody conjugated with Alexa Fluor 524 overnight at 4 °C. After washing, brains were mounted using Vectashield (Thermo Fisher Scientific Inc.) and images were acquired using confocal microscopy (Carl Zeiss Microscopy).

2.6. Brain histology

The *Drosophila* heads that were fixed in 4% paraformaldehyde were embedded in paraffin blocks and sectioned at a thickness of 6 μm. These sections were mounted on slides and stained using hematoxylin and eosin. Quantification of the neurodegeneration was performed as mentioned by Lijima et. Al (2008) [14].

2.7. Climbing assay

Around 20 flies of each genotype were placed in a 50-mL glass mass cylinder and gently tapped to the bottom. The number of flies that climbed to the top was counted after 10 s. The data were acquired from three independent sets and then analyzed and plotted.

2.8. Data analysis

The longevity data were used to generate Kaplan-Meier survival plots, and median lifespan was calculated. Statistical significance between the genotypes was analyzed using Log-Rank tests and the Prism software. For the data on the CR, sleep parameters, sleep deprivation and sleep recovery were analyzed using a 2-way analysis of variance with the main factors of genotype and light condition. The number of vacuoles and pTau intensity of the western blots were analyzed using a 2-way repeated analysis of variance. P values less than 0.05 were considered significant, and the data are presented as mean ± standard error of the mean.

3. Results

3.1. Accumulation of hTau negatively affected lifespan

Studies of hTau have shown that the accumulation of hTau in fly brains shortens their lifespan and increases neurodegeneration in *Drosophila* [18]. Two types of human tau (hTau) protein were overexpressed: wild-type hTau (hTau^{WT}) and mutant hTau, in which Arg406 was replaced by Trp (hTau^{RW}). Accumulation of hTau proteins in the brain were confirmed with both immunohistochemistry (Fig. 1A) and western blotting (Fig. 1B). Flies overexpressing hTau^{WT} exhibited the distinct rough eye phenotype under control of either the *Elav-GAL4* or the *GMR-GAL4* (Fig. 1C). The rough eye phenotype was exacerbated in hTau^{RW} flies overexpressed both in neurons and the eye. Flies overexpressing hTau^{WT} or hTau^{RW}, in neurons showed significant shorter lifespans compared to controls ($p < 0.001$, Fig. 1D). The median lifespan (20 days) of the hTau^{RW}-overexpressing flies was significantly shorter than that (39 days) of the hTau^{WT}-overexpressing flies ($p < 0.001$, Fig. 1D). Furthermore, these results suggested that the neuronal overexpression of the *hTau* gene enhanced the formation of pTau and reduced lifespan in *Drosophila*.

Please cite this article in press as: M. Kim, et al., Short-term exposure to dim light at night disrupts rhythmic behaviors and causes neurodegeneration in fly models of tauopathy and Alzheimer's disease, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.12.021