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Research article

Chronic sleep fragmentation exacerbates amyloid β deposition in Alzheimer's disease model mice

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HIGHLIGHTS

- Sleep fragmentation resembling that in AD patients were induced in AD model mice.
- Sleep fragmentation exacerbated amyloid β (Aβ) deposition in the AD mouse brain.
- The severity of Aβ deposition correlated with the extent of sleep fragmentation.

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ABSTRACT

Sleep fragmentation due to intermittent nocturnal arousal resulting in a reduction of total sleep time and sleep efficiency is a common symptom among people with Alzheimer's disease (AD) and elderly people with normal cognitive function. Although epidemiological studies have indicated an association between sleep fragmentation and elevated risk of AD, a relevant disease model to elucidate the underlying mechanisms was lacking owing to technical limitations. Here we successfully induced chronic sleep fragmentation in AD model mice using a recently developed running-wheel-based device and demonstrate that chronic sleep fragmentation increases amyloid β deposition. Notably, the severity of amyloid β deposition exhibited a significant positive correlation with the extent of sleep fragmentation. These findings provide a useful contribution to the development of novel treatments that decelerate the disease course of AD in the patients, or decrease the risk of developing AD in healthy elderly people through the improvement of sleep quality.

1. Introduction

The entire function of sleep remains to be elucidated despite mounting evidence demonstrating its importance for various organisms including humans [1]. Among the emerging role of sleep is the maintenance of protein homeostasis (proteostasis) in the brain through various intra- and extracellular processes [2]. Acute sleep deprivation induces the upregulation of BiP/GRP78, a classical marker and a key regulator of the endoplasmic reticulum stress response [3], which triggers multiple pathways to protect cells from protein toxicity [2]. The clearance efficiency of the glymphatic system, which eliminates potentially neurotoxic proteins from extracellular space in the central nervous system, is greatly enhanced during sleep [4]. A growing body of evidence suggests the possibility that sleep disturbance (SD) leads to altered proteostasis in the brain [2].

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The disruption of proteostasis in the brain strongly affects the onset and progression of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) and leads to abnormal protein accumulation in the brain, which is the pathological hallmark of neurodegenerative diseases such as amyloid β (Aβ) and tau deposition in AD and α-synuclein deposition in PD [5]. Recent studies have suggested a bidirectional relationship between disruption of proteostasis and SD in neurodegenerative diseases [6]. Disruption of proteostasis results in abnormal protein accumulation and neuronal loss in a number of brain regions including those involved in sleep-wake regulation such as basal forebrain, hypothalamus and brainstem [6–8], and hence induces SD. These regions are susceptible to AD or PD pathology from the early stage of the disease, consistent with the finding that SD occurs early in the clinical course of the patients. SD, in turn, alters proteostasis and aggravates the abnormal aggregation and accumulation of proteins in the brain [6].

Nearly half of all AD patients suffer from SD, and one of the most common sleep-related symptoms among AD patients is sleep fragmentation due to intermittent nocturnal arousal resulting in a reduction of total sleep time and sleep efficiency [9,10]. These changes in the sleep architecture are similar to age-related changes in the sleep architecture of elderly people [9,10]. Importantly, sleep fragmentation exacerbates the risk of developing AD in individuals harboring a genetic risk factor of AD [11]. Furthermore, sleep fragmentation in elderly people with normal cognitive function is associated with an elevated risk of incident AD [12]. Multiple AD mouse models have been used to investigate the mechanisms underlying these epidemiological findings, and have demonstrated the exacerbation of AD pathology under SD [13–15]. In these studies, SD was induced either by intermittent tactile stimuli resulting in acute total sleep deprivation [13], the platform-over-water technique resulting in chronic elimination of rapid-eye-movement (REM) sleep and a reduction of slow wave sleep [13,14], or alteration of the light-dark cycle resulting in the interruption of regular circadian activity [15]. The patterns of resultant SD in these studies, however, are discrepant from SD observed in AD patients.

In the present study, we utilized a recently-developed running-wheel-based device [16] to induce chronic sleep fragmentation and reduced delta power of non-REM sleep in AD model mice. This pattern of SD has similar characteristics to those observed in AD patients [9,10]. We revealed that chronic sleep fragmentation exacerbates Aβ deposition, and that the extent of sleep fragmentation shows significant positive correlation with the severity of Aβ pathology. These findings are in line with the findings of previous human studies [11,12] and hence provide a useful contribution to elucidating the underlying pathomechanism of these studies [11,12] and to developing novel treatments that decelerate the disease course of AD in the patients, or decrease the risk of developing AD in healthy elderly people through the improvement of sleep quality.

2. Materials and methods

2.1. Animals and induction of sleep disturbance (SD)

Animal care, handling, and the experimental procedures were approved by the Animal Ethics Committee of the National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan. APPswe/PSEN1dE9 (APP/PS1) mice were obtained from the Jackson Laboratory (MMRRC 034832-JAX) and maintained on a C57BL/6J background under a 12 h light–12 h dark cycle (LD 12:12; lights on at Zeitgeber time [ZT] 0) with food and water provided *ad libitum*. At 9 weeks of age, when these mice have not developed abnormal sleep-wake cycles [17,18], female mice were assigned to one of three groups (Fig. 1A, day −9). Mice in the normal cage (NC) group were housed individually in a plastic cage with paper bedding from day −9 to 27. The remaining mice were transferred to plastic cages equipped with running wheels and paper bedding (referred to as a "wheel cage"; SW-15, Melquest, Toyama, Japan) on day −9 for acclimation, and were housed individually. On day 0, when the daily wheel-running activity reached a plateau, each mouse was either transferred to an "SD cage" (SW-15-SD, Melquest) with paper bedding in the bottom tray (SD group), or remained in the wheel cage with paper bedding (WC group). On day 27, all mice were sacrificed for immunohistochemical analyses. Of the 25 mice used in this study, 6, 4 and 12 mice were assigned to NC, WC and SD groups, respectively, and 3 mice at 9 weeks of age were analyzed as a baseline control.

2.2. Recording and analyses of wheel-running activity

The number of running-wheel revolutions was recorded at 1-min intervals using a counter interface (CIF-III, Melquest) and CIF-3WIN software (Melquest) from day −9 to 27 for all mice in the WC and SD groups. The wheel count data were accumulated into 15-min intervals and displayed as double-plotted actograms using CIF-3WIN. In Fig. 1B and C, the scale of the vertical axis is adjusted to best visualize the activity during ZT0-12, resulting in saturation of the histogram during ZT12-0. In Supplementary Fig. 1A and B, the vertical scale of the vertical axis is adjusted to avoid saturation during ZT12-0. For activity analyses, inactive and active bouts were defined as a successive period of time in which the number of wheel counts per minute remained equal to zero, or greater than zero, respectively. The total number of active bouts, the duration of each inactive bout and the total amount of time spent inactive (defined as the total length of inactive bouts) during the light phase, and the total activity (defined as the total number of wheel counts during the light and dark phase from day −9 to 27) were calculated using R software (version 3.2.5; R Computing for Statistical Computing, Vienna). The pre-SD period was defined as the final 2 days of acclimation (day −2 and −1), when the daily wheel-running activity had reached a plateau. The post-SD period was defined as day 7 to 20, when the effect of SD cage on the daily wheel-running activity of each mouse in the SD group had reached a plateau.

2.3. Tissue preparation and immunohistochemistry

Mice were deeply anesthetized and transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) followed by 10% neutral buffered formalin (NBF). Brains were dissected out, post-fixed in 10% NBF at 4 °C overnight, embedded in paraffin and coronally sectioned at a thickness of 5 μm. Immunohistochemistry for amyloid plaques were performed as described previously [19] with slight modifications. Sections were deparaffinized, antigen retrieved with 70% formic acid for 30 min at room temperature, blocked with 5% horse serum in 1% bovine serum albumin in PBS containing 0.2% Tween 20, and incubated with 6E10 antibody (SIG-3932, Covance, Princeton, NJ, USA) at 4 °C overnight. Sections were then incubated with biotinized horse anti-mouse antibody (Vector Laboratories, Burlingame, CA, USA) followed by streptavidin conjugated with horse radish peroxidase (DAKO, Glostrup, Denmark) in PBS. The sections were colorized using 3,3'-diaminobenzidine tetrahydrochloride (Dojindo Laboratories, Kumamoto, Japan) solution containing 0.03% hydrogen peroxide, counterstained with hematoxylin and examined with bright-field microscopy (BZ-X710, Keyence, Osaka, Japan). Aβ plaques in the whole brain section was quantitatively analyzed as previously described [20] using dedicated software (Hybrid Cell Count System BA-H3C, Keyence), with the experimenter (H.Y.) blinded to the experimental group. The