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Research article

Continuous theta burst stimulation facilitates the clearance efficiency of the glymphatic pathway in a mouse model of sleep deprivation

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HIGHLIGHTS

- Sleep deprivation impairs the function of the glymphatic system.
- cTBS accelerates the solute clearance rate in the glymphatic pathway.
- cTBS improves the behavioral disorders in sleep deprivation mice.
- cTBS restores the polarization dysfunction in sleep deprivation mice.

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ABSTRACT

Sleep deprivation (SD) is a common condition associated with a variety of nervous system diseases, and has a negative impact on emotional and cognitive function. Continuous theta burst stimulation (cTBS) is known to improve cognition and emotion function in normal situations as well as in various types of dysfunction, but the mechanism remains unknown. We used two-photon in vivo imaging to explore the effect of cTBS on glymphatic pathway clearance in normal and SD C57BL/6J mice. Aquaporin-4 (AQP4) polarization was detected by immunofluorescence. Anxiety-like behaviors was measured using open field tests. We found that SD reduced influx efficiency along the peri-vascular space (PVS), disturbed AQP4 polarization and induced anxiety-like behaviors. CTBS significantly attenuated the decrease in efficiency of solute clearance usually incurred with SD, restored the loss of AQP4 polarization and improved anxiety-like behavior in SD animals. Our results implied that cTBS had the potential to protect against neuronal dysfunction induced by sleep disorders.

1. Introduction

Sleep plays a critical role in the glymphatic clearance of brain wastes because the interstitial space increases by about 60% during sleep, thus significantly enhancing the clearance rate of the by-products generated in the brain [1]. Insufficient sleep or sleep deprivation (SD) is considered a universal phenomenon in modern life [2]. Approximately 36% of adults in the USA suffer from poor sleep [2]. SD is closely associated with Alzheimer's Disease (AD), cerebral amyloid angiopathy (CAA) and other neuropsychiatric disorders [2], leading to cognitive deficits [3] and anxiety [4] However, the mechanism of SD-mediated neuronal dysfunction remains elusive and there are no effective methods to manage these consequences.

The by-products generated by high level of metabolic activity of neurons in the central nervous system require rapid elimination [5]. The glymphatic system plays an essential role in the clearance of brain metabolic wastes such as soluble amyloids [6]. In

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the glymphatic system, cerebrospinal fluid (CSF) rapidly enters the brain parenchyma from the subarachnoid space via the Virchow-Robin spaces, along PVS, and exchange with the interstitial fluid (ISF) compartment [6]. Through the exchange between the CSF and ISF, waste products are eliminated from the brain [5]. Failure of the glymphatic pathway leads to accumulation of brain wastes such as amyloid plaque deposition, and is believed to contribute to brain disorders including AD.

The transport of CSF into the brain parenchyma is facilitated by astroglial aquaporin-4 (AQP4) water channels which are highly polarized to perivascular endfeet and ensheathe the brain vasculature in normal situations [7] and plays an essential role in maintaining the normal function of the glymphatic pathway, as evidence shown that both a lack of AQP4 and a loss of AQP4 polarity are associated with a significant reduction in the clearance rate of the glymphatic pathway [8].

Currently, continuous theta burst stimulation (cTBS), a pattern of a repetitive transcranial magnetic stimulation (rTMS), has been shown to enhance sleep efficacy and reduce sleep-associated emotional and cognitive dysfunction [9]. However, the underlying mechanism is not yet clear, and the potential effect of cTBS on the glymphatic system has not been studied. Therefore, the objective of this study was to assess the protective effects of cTBS on a mouse model of SD, and explore the possible mechanism.

2. Materials and methods

2.1. Animals

All experiments were approved by the Committee of Sun Yat-sen University (Guangzhou, China) on the Care and Use of Animals. We used a total of 108 male C57BL/6 mice (aged 6–8 weeks; weighing 20–25 g) in this experiment. The mice were randomly divided into four groups: natural sleep with sham cTBS group (NS), natural sleep with real cTBS group (NS + cTBS), sleep deprivation with sham cTBS group (SD), and sleep deprivation with real cTBS group (SD + cTBS). Mice were housed five per cage and were exposed to a 12-h light/dark cycle with free access to food and water. 1% pentobarbital (50 mg/kg) were intraperitoneally injected to anesthetize the mice, and animals were kept at room temperature (22 °C) throughout all experiments. Body temperature was kept constant at 36.8 °C using a feedback-controlled heating pad (RWD Life Science company, Shenzhen, China) [10].

2.2. Sleep deprivation (SD) protocol

The mice in the experimental groups were sleep deprived for 48 h with a modified multiple platform method [11]. The apparatus consisted of 12 circular platforms placed in a water tank (40 × 40 × 40 cm). Each of the platforms had a diameter of 3.5 cm and the surfaces were 1 cm above the water. Six mice were placed on the platforms so that they could freely move from one platform to another and if any one of the mice fell asleep, it would fall into the water and wake up. Food and drinking water were available to the mice and the room temperature was kept at 22 °C [12]. NS mice were kept in their home cages in the same room.

2.3. Performance of continuous theta burst stimulation (cTBS)

The cTBS was performed immediately after 48 h of SD with a focal figure-of-eight shape coil (Each loop had a diameter of 3.5 cm) connected to a Yiruide CCY-IA magnetic stimulation device (Wuhan Yiruide Medical Equipment New Technology Co. Ltd., Wuhan, China), cooling in liquid nitrogen. Each cTBS conditioning session consisted of 3-pulse bursts at 50 Hz, repeated at 5 Hz. Thus, 600 pulses were applied to the cranium of the mice, with intervals of 0.02 s, for a total of 40 s for each mouse at a 20% threshold of maximum output intensity. During stimulation, the paws of the mice were restrained and the coil was placed flat on the scalp [13]. For the sham condition, the coil was placed at a perpendicular angle on the scalp of paw-restrained mice [14].

2.4. The open field test

To evaluate locomotor activity and anxiety-like behavior, we performed an open field test in all four groups of mice. An apparatus consisting of a 40 × 40 cm white plywood arena, surrounded by 50 cm high wooden walls, was used [15]. The top of this arena was open and the arena was under a tracking of a camera. The mice were taken out of their home cages and carefully put into the center of the arena for 1 min, to allow them to adapt to the strange environment. Next, they were returned to their home cages for several minutes and then put back into the arena for 10 min. The total distance travelled was recorded [16]. Before each mouse was tested, the arena was cleaned with 5% alcohol solution to clear cues left behind by the last animal and then left for minutes [17].

2.5. Two-photon in vivo imaging

Mice were anesthetized with 1% pentobarbital (50 mg/kg) by an intraperitoneal injection. A cranial window measuring 2 × 2 mm was created with a micro drill over the right parietal cortex (−2 mm bregma, 1.7 mm lateral) as described previously [18] until the skull to an approximate thickness of 20–30 μm to visualize cerebral vasculature without opening the skull. Then a domestic metal plate was glued at the edge of the cranial window to fix it. The location of the cranial window was determined using a stereotaxic frame (RWD Life Science Company). Artificial cerebrospinal fluid (ACSF) was perfused throughout the surgical procedures to keep the cranial window moist. Rhodamine (70 kDa, FD20, Sigma, Germany) was injected through the tail vein to image the blood flow, and to visualize the CSF flux, 20 μl fluorescein isothiocyanate (FITC)–dextran (70 kDa, 0.2 mg/ml; FD20, Sigma) was slowly injected into the cisterna magna with a syringe pump at a speed of 2 μl/min. Immediately after the injection, the mice were imaged with a two photon laser scanning microscope (Leica, Germany) with 25 magnification (0.95 numerical aperture, NA) water immersion lens to obtain a high-resolution image. The excitation wavelength was 800 nm for both FITC and rhodamine. We photographed the cerebral vasculature with 512 × 512 pixel frames, with a depth of about 200 μm from the surface, with 5-μm z-steps at 10 min, 15 min, and 20 min, and repeated the operation every 10 min for 1 h. The three-dimensional stack of images was obtained with a 10 magnification air objective (0.12 NA) throughout the cranial window [10]. The pictures of 100 μm depth from the surface were captured with 1024 × 1024 pixel frames. All data acquisition and laser scanning were under control of Leica LAS AF 2.5 software. Images were captured with 2-channel detector, with an emission filter of 525/50 and 585/50 nm on TCS SP5 MP System (Leica Microsystems, Inc.) [19].

2.6. Immunofluorescence

Immediately after in vivo imaging, the mice were sacrificed using perfused 0.9% saline, followed by 4% paraformaldehyde. The whole brains of the mice were removed and soaked in 4% paraformaldehyde for 12–18 h, and then gradient dehydrated in 20% and 30% sucrose for one day each at 4 °C. Brain tissues were sectioned consecutively into 10 μm thick sections with a cryostat microtome (Leica). For every brain, 60 sections were collected and stored at a temperature of −40 °C.