Sleep deficiency exacerbates age-related decline in brain drainage and clearance in mice

V. Adushkina[1*], A. Terskov[1], D. Zlatogorskaya[1], A. Evsyukova[1], N. Navolokin[2], A. Shirokov[3]

[1]Saratov State University, Saratov, Russia
[2]Saratov State Medical University, Saratov, Russia
[3]Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia
[*]adushkina.2000@mail.ru

Meningeal lymphatic vessels (MLV) drainage of the brain plays a key role in maintaining homeostasis and removing toxic metabolites. With age, the function of the MLV naturally deteriorates, which contributes to the accumulation of metabolic products in brain tissues. However, in real life, aging rarely occurs in isolation — it is often accompanied by additional negative factors, among which one of the leading places is occupied by chronic sleep disorders. It is known that sleep is a natural time for activating brain drainage. It is logical to assume that such two processes as age-related sleep disorders and decreased brain drainage are interrelated, which, however, remains poorly understood.

The aim of the research was to study the effects of acute and chronic sleep deficiency on brain drainage in mice of different ages.

The studies were conducted on male C57BL/6 mice (aged 3, 12, and 24 months). To simulate sleep deficiency, the "What is it?" reflex method was used by providing a new object to the cage every 3 hours, which caused arousal in mice and activation of research activities. For acute sleep deficiency, the method was used for 24 hours, for chronic - for 10 days, where sleep was excluded every day from 17:00 to 20:00, which is associated with the preference of mice to sleep at this time of day. EEG recordings were used to assess sleep and wakefulness. To study the effect of sleep deficiency on brain drainage, confocal ex vivo analysis of the distribution of fluorescein isothiocyanate carboxymethyl dextran (FITCD, 70 kDa, Sigma, 5 µl, intraventricular injection at a rate of 0.1 µl/min) in the dorsal and ventral parts of the brain was performed. To analyze the effects of sleep deficiency on the excretion of metabolites from the brain, the ELISA method was used to quantify the metabolites of neurons in brain structures.

The results revealed an expected age-related decrease in brain drainage. Indeed, the intensity of the FITCD signal in 24-month-old mice compared with 3- and 12-month-old mice was 2.2 times ($p<0.001$) and 2.7 times ($p<0.001$) lower in the ventral part of the brain and 1.7 times ($p<0.01$) and 0.6 times ($p<0.01$) is lower in the dorsal parts of the brain. Chronic but not acute sleep deficiency was accompanied by a decrease in brain drainage, and it was more pronounced in old mice than in young and middle-aged animals. Thus, after chronic sleep deprivation, compared with control, the intensity of the FITCD signal in the ventral and dorsal parts was lower in 24-month-old mice by 7.8 times ($p<0.01$) and 5.6 times ($p<0.01$), in 12-month-old mice by 3.5 times ($p<0.001$) and 2.2 times three times ($p<0.001$), 1.7 times ($p<0.001$) and 1.7 times ($p<0.001$) in 3-month-old mice.

Decreased brain drainage caused by chronic sleep deficiency led to the accumulation of neuronal metabolites such as beta-amyloid and tau protein in mice of all ages. However, these changes were statistically significant only in older mice. Thus, the level of beta-amyloid in the brain

of 24-month-old mice was 18.30 ± 2.26 pg/g of protein versus 11.65 ± 1.25 pg/g of protein; 123.35 ± 2.26 pg/g of protein versus 92.40 ± 3.41 pg/g of protein.

Overall, the results revealed that chronic sleep deficiency worsens the age-related decline in brain drainage and the excretion of toxic metabolites from mouse brain tissues. These findings suggest that maintaining adequate sleep in old age is important for maintaining the drainage function of the brain and preventing accelerated aging of neurons.

Keywords: drainage, chronic sleep deprivation, meningeal lymphatic vessels.

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