Acta Pharmaceutica Sinica B 2025;15(2):1193−1195

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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HIGHLIGHT

Neuronal activities drive brain waste clearance through the glymphatic system

KEY WORDS

Alzheimer disease;
Neuron;
Glymphatic system;
Cerebrospinal fluid perfusion;
Brain clearance

Neurons are the main organizers of neurological activities in the brain. However, its role remains largely unknown in regulating waste clearance in the brain. This issue is addressed in a recent study published in *Nature* on neuronal dynamics in control of waste removal through cerebrospinal fluid perfusion[1]. Brain contains billions of neurons that are connected through synapses for precise spatial coordination and high-speed operation in support of functions like thoughts, emotions, and body movements in a dynamic manner. Brain consumes a substantial amount of biological fuel to support the neuron activities, which is associated with production of a considerable volume of metabolic wastes. Accumulation of the large molecule wastes, such as amyloid-β (Aβ) and phosphorylated tau proteins, are able to cause neuron damages for an increased risk of AD. Clearance of the wastes is a strategy in preservation of neuron functions in the prevention of brain diseases. The new study suggests that neurons dominate the waste clearance activity in the brain by promoting cerebrospinal fluid perfusion during sleep[1].

The central lymphatic system is crucial for clearing large molecule wastes from the brain. Despite many advances in the central lymphatic system[2], substantial gaps remain in the regulation of the waste clearance function. Previous reports suggest that CSF flows through the perivascular space and the movement is driven, at least in part, by arteriole pulsation. The vessel diameter increases in response to sensory stimuli, likely involving slow contraction and relaxation of smooth muscle in the vessel walls (vasomotion). Arterial pulsation decreases with aging and AD, contributing to the reduced CSF-ISF exchange in the waste clearance function[3]. However, the glymphatic system is not driven by vascular pulsation due to the lack of a significant pressure gradient between the para-arterial and para-venous spaces to drive convection flow through the brain parenchyma[4]. Consequently, the mechanisms underlying fluid perfusion for waste clearance in the high-resistance brain parenchyma remain largely unknown.

Previous studies have suggested that neurons may play a dominant role driving waste removal through the glymphatic system, a factor that had been previously overlooked[5]. Researchers observed a 60% increase in the interstitial space of the cerebral cortex during natural sleep or anesthesia, which accelerates amyloid-β clearance to nearly twice the rate observed during wakefulness, suggesting that CSF perfusion is regulated by cerebral rhythms[6]. In addition, electroencephalography (EEG) studies have shown that neuronal activity can predict hemodynamics and CSF oscillations during sleep in humans, with high delta EEG signals correlation with increased glymphatic influx across different brain states and anesthetic treatment[7]. However, these correlations do not definitively prove that neuronal activity drive the fluid flow, leaving the hypothesis controversial[4].

In the new study, Xie and colleagues confirmed the potential interdependence between neuronal activity and fluid dynamics in brain tissue by placing a linear electrode array in the hippocampus to monitor neuronal action potentials and ion dynamics, while concurrently acquiring EEG and electromyography (EMG) recordings to assess global brain states[1]. They observed that, compared to small and irregular fluctuations in the hippocampus during wakefulness, large-amplitude delta ionic waves (0.5−4 Hz) and highly rhythmic theta oscillations (6−10 Hz) originated in the hippocampus during non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, respectively. Remarkably, their recordings of neuronal activity and fluid dynamics revealed that

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.11.005

individual neuron spikes consistently synchronized with larger fluctuations in field potentials during sleep, suggesting that coordinated action potentials from neuronal networks may generate large-amplitude, rhythmic ionic waves in the interstitial fluid (ISF).

To determine whether these field potential dynamics were strictly neuronally driven, the researchers employed chemogenetic toolkits to achieve specific inhibition of hippocampal neuronal activity through administration of uPSEM792[1]. Upon inhibition of neuronal activity, the ionic waves in the interstitial space were flattened. Using fluorescent molecular tracing and magnetic resonance imaging (MRI), the researchers observed a dramatic reduction of CSF perfusion in the hippocampus during sleep following inhibition of neuronal spike firing, suggesting rhythmic neuronal activity is critical for CSF perfusion.

Furthermore, they addressed the unresolved question of whether neuronal activity influenced the brain's clearance of metabolites[1]. When the low molecular weight tracer (dextran-Texas Red, 3kda) was infused with CSF into the brain parenchyma, its gradual efflux from the brain parenchyma was significantly impaired by chemogenetic inhibition, resulting in a marked increase in trapped molecules within the hippocampus. This finding indicates that neuronal activity may dominate the clearance of molecules from the parenchyma.

Conversely, by utilizing a newly developed transcranial optogenetic technique to generate slow and theta waves in the ISF, the researchers found that these synthetic waves enhanced cerebral CSF-ISF perfusion, further supporting the hypothesis that rhythmic neuronal activity during sleep is crucial for CSF-to-ISF perfusion and waste clearance in the brain[1]. The transcranial optogenetics introduced in this study enables the generation of synthetic ionic waves in the interstitial space without invasive fiber penetration, thereby avoiding acute damage to the brain parenchyma[8]. These findings and techniques offer new avenues for treating diseases associated with waste accumulation in the brain, such as Alzheimer's disease, by facilitating the artificial removal of metabolic byproducts.

In summary, Xie and colleagues demonstrated that neurons may function as miniature pumps to drive clearance of metabolic wastes through the brain's glymphatic system[1]. However, several questions remain. Transcranial optogenetics was performed solely in the hippocampus, leaving it unclear whether slow wave and theta rhythms can sufficiently promote CSF infiltration into other brain parenchymal regions, or if specific rhythmic wavelengths are required across different areas. Additionally, transcranial optogenetics was tested at 1 Hz delta or 8 Hz theta rhythm, whereas sleep-related brain oscillations span a broader frequency range, including delta (0.5−4 Hz), theta (6−10 Hz), spindle (12−15 Hz), and ripple (140−200 Hz) rhythms. Notably, previous studies have shown that sensory stimulation with 40 Hz gamma rhythms increases neuronal activity to promote clearance of amyloid β in the brain[9]. However, the effects of varying rhythmic oscillations have yet to be examined. Furthermore, in verifying whether neuronal activity affects metabolite clearance, only a low-molecular-weight tracer was utilized, preventing conclusions about whether different rhythmic oscillations can clear varying molecular weights of waste, or whether neuronal activities modulate the strength of this clearance process, particularly under varied environmental or pathological conditions.

In addition, there is another recent study on CSF, in which CSF is found to transport substances from the central nervous system (CNS) to the peripheral nervous system (PNS)[10]. Previously, CSF was thought to circulate only within the brain and spinal cord, with the PNS having its own independent nutrient supply. Through gold nanoparticle tracking and high-resolution electron microscopy, researchers demonstrated that CSF flows between the CNS and PNS, suggesting a more unified system for nutrient delivery and waste clearance across the entire nervous system. The effect of ionic waves generated by neuronal activity on CSF flow within this integrated system remains unclear. Nonetheless, neurodegenerative diseases like Alzheimer's and Parkinson's may involve dysfunctions in both central and peripheral nerves. Modulating CSF flow or delivering therapeutic agents directly into the CSF could potentially enhance treatment effectiveness for conditions like peripheral nerve injuries by more precisely targeting peripheral nerves. Furthermore, the study identified a "size limitation" in CSF flow by tracking various sizes of gold nanoparticles, offering valuable insights for future drug design. These findings open a new chapter in nervous system research, paving the way for more-precisely targeted therapeutic strategies that leverage CSF flow mechanisms to treat neurological diseases.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Grant No. 32271220).

Author contributions

Lin-jian Wang drafted the manuscript. Xiwen Ma and Jianping Ye provided the idea and revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Received 30 August 2024
Received in revised form 6 November 2024
Accepted 7 November 2024