Abstract of Doctoral Dissertation

Degree requested: Doctor of Life Science Applicant's name YANG GANGSHENG

Title of Doctoral Dissertation

Development of a Closed-loop Transcranial Ultrasound Neuromodulation Technology for a Deep Brain Region with Genetic Enhancement of Ultrasound Sensitivity of Neurons.

深部脳領域における神経細胞の超音波感受性を遺伝子工学的に増強したクローズドループ経頭蓋超音波神経調節技術の開発.

Introduction

Noninvasive neuromodulation techniques have attracted increasing attention as alternatives to invasive approaches such as deep brain stimulation (DBS), which require surgical implantation and carry associated risks and costs. However, widely used noninvasive methods, including transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (TES), are limited by shallow penetration depth and poor spatial resolution, making it difficult to selectively modulate deep brain structures.

Transcranial focused ultrasound stimulation (tFUS) offers a unique advantage in that acoustic energy can be focused deep within the brain with millimeter-scale precision. Despite this potential, the efficacy of ultrasound neuromodulation in deep brain regions remains constrained by the relatively low intrinsic sensitivity of neurons to mechanical stimulation. Sonogenetics strategies, which genetically enhance neuronal sensitivity to ultrasound through the expression of mechanosensitive ion channels, have therefore been proposed to lower activation thresholds and improve the efficiency of neuromodulation.

The medial septum (MS) is a deep midline brain structure that plays a central role in regulating hippocampal network activity and theta oscillations via its dense projections to the hippocampus. Its anatomical position also makes it suitable for transcranial ultrasound targeting. In this study, mechanosensitive ion channels were selectively expressed in MS neurons to enhance ultrasound responsiveness. In addition, a closed-loop tFUS system was developed to enable real-time, brain activity–dependent ultrasound neuromodulation in freely moving animals.

Sonogenetic enhancement of medial septal neurons and modulation of hippocampal activity.

To enhance ultrasound-induced activation of a deep brain region, the mechanosensitive ion channels MscL-G22S and TRPC6 were selectively overexpressed in medial septal neurons using adeno-associated viral vectors, while control animals received vectors expressing fluorescent proteins alone (tdTomato or eYFP) without mechanosensitive channels. Neuronal responses to transcranial focused ultrasound stimulation were evaluated using in vivo electrophysiological recordings and c-Fos immunohistochemistry.

Medial septal neurons expressing MscL-G22S exhibited clear and time-locked increases in firing activity in response to ultrasound stimulation, whereas control neurons showed minimal modulation under the same stimulation parameters. Quantitative peri-stimulus time histogram analysis demonstrated that the proportion of ultrasound-responsive neurons was significantly higher in the MscL-G22S group at stimulation pressures of 0.25 and 0.35 MPa. While MscL-G22S expression markedly increased the likelihood of neuronal activation, the maximum firing rates of responsive neurons were not significantly different from controls, indicating that MscL-G22S primarily lowered the activation threshold rather than altering intrinsic firing properties.

MscL-G22S expression was also associated with shorter response latencies and more time-locked neuronal firing in response to ultrasound stimulation. A larger fraction of medial septal neurons displayed short-latency responses (< 50 ms) compared with control animals. These electrophysiological findings were supported by c-Fos immunostaining, which revealed a significant increase in the number of activated neurons in the medial

septum following ultrasound stimulation in MscL-G22S–expressing rats. In contrast, overexpression of TRPC6 produced only modest and statistically non-significant effects in both electrophysiological responses and c-Fos expression under the same stimulation conditions.

Given the established role of the medial septum in controlling hippocampal network dynamics, the downstream effects of medial septal ultrasound stimulation were examined. Four to five weeks after viral transduction in the medial septum, hippocampal neuronal activity was recorded during transcranial ultrasound stimulation targeted to the MS.

In animals expressing MscL-G22S in the medial septum, ultrasound irradiation elicited mild but clearly time-locked firing responses in hippocampal CA1 neurons, whereas responses in control animals remained weak. The proportion of ultrasound-responsive neurons in the CA1 was significantly higher in the MscL-G22S group, and response latency was significantly shorter than in controls. In contrast, maximum firing rates of responsive CA1 neurons did not differ between groups. Neurons in the CA3 region exhibited minimal responses in both groups, with no significant differences in response probability or response timing. These results indicate that sonogenetic enhancement of medial septal neurons preferentially modulates hippocampal CA1 activity, consistent with known anatomical connectivity.

Closed-loop transcranial focused ultrasound stimulation in freely moving animals

A closed-loop transcranial focused ultrasound stimulation system was developed to allow real-time neuromodulation in freely moving rats. This system integrated real-time neural signal acquisition with automatic triggering of ultrasound stimulation upon detection of epileptiform activity.

The closed-loop system was evaluated using a hippocampal kindling epilepsy model. The system reliably detected seizure-related neural activity and delivered precisely timed ultrasound stimulation to the medial septum. However, under the stimulation parameters tested, closed-loop ultrasound stimulation did not significantly reduce seizure duration compared with control conditions, suggesting that further optimization of stimulation timing or parameters is required. Nevertheless, these experiments demonstrate the technical feasibility and stability of adaptive, real-time ultrasound neuromodulation in freely moving animals.

Conclusion

This study demonstrates that transcranial focused ultrasound stimulation combined with sonogenetic strategies enables reliable, noninvasive modulation of a deep brain region. Overexpression of the mechanosensitive ion channel MscL-G22S substantially enhanced the sensitivity and temporal precision of medial septal neurons to ultrasound stimulation and enabled effective modulation of downstream hippocampal CA1 activity, whereas TRPC6 provided only limited enhancement under identical conditions. In addition, a closed-loop ultrasound neuromodulation system was successfully developed for freely moving animals, establishing a technical foundation for real-time, brain activity–dependent ultrasound control. Together, these findings provide a basis for future development of noninvasive, adaptive ultrasound neuromodulation approaches targeting deep brain circuits involved in neurological and psychiatric disorders.