1. No.
   2023G014

2. Title
   Regulation of Calcium Oscillations and Clock Genes by Nicotine in 3D Neuronal Spheroid Culture

3. Period
   2023-2025

4. Summary
   In this study, we established a high-throughput (HTS)-compatible Ca^{2+} oscillation assay based on synaptic network formation in densely cultured primary rat neurons and iPS-derived neurons. In 3D spheroid neurons using the FDSS-7000 (Hamamatsu Photonics), we observed a time-dependent increase in the frequency and a decrease in the amplitude of Ca^{2+} oscillations under ischemic stress. Inhibition of NMDA and AMPA receptors suppressed Ca^{2+} oscillations, while inhibition of NR2A/NR2B exhibited opposite effects. We identified differences in responsiveness depending on nicotinic receptor subtypes, such as an increase in frequency upon α7 nAChR stimulation. We constructed a Ca^{2+} oscillation database using the LOPAC1280 library and classified it into 10 clusters using UMAP. Furthermore, we established a human iPS-derived neuron–astrocyte co-culture system and a stable Ca^{2+} oscillation system. By integrally applying multidimensional parameter analysis, UMAP, Bayesian bootstrap, and MDS, we accurately identified characteristic response patterns for each drug and classified their pharmacological effects. We are analyzing the pharmacological properties and neuroprotective mechanisms of various nicotine-related compounds. Additionally, using AAV-mediated gene delivery of SIK3, which is associated with sleep, and SIK3T mice, we investigated the relationship between Ca^{2+} oscillations and clock genes.