Abstract

Exploring the cell and neurons is important for better understanding the physiology of the body system. This investigation is elaborated to convince the recording of biological work in non-invasive techniques with various micro- or nano-electrodes. In these ways, electrophysiology has become one of the best biological methods in ongoing research. Voltage-gated calcium channels are highly selective for calcium and have a broad range of animating or deactivating features. Based on their threshold of voltage-dependent facilitation, they are classified as high and low-voltage-activated channels. Somatic calcium can be recorded to show the activity of action potential. Somatic calcium can trigger gene transcription. N-methyl-D-aspartate is called an ion-based glutamate receptor and can mediate postsynaptic calcium ions in the cortex or pyramidal neurons. Calcium imaging is generally operated for the monitoring of interconnected neurons such as analyzing the circuitry in the cortex. This idea is also applied to recognize synaptically bridged neurons.

Previously, it was the major problem that cell-type specific recording was not possible but with time optical-approach-type electrophysiological techniques now can do this job. This was done by the genetically encoded protein which only detects the specific cell which is matched with their cell type-specific promoter. But it is still a problem for the prob/sharp microelectrode-based electrophysiological techniques to determine cell-type-specific recording. So, using the properties of a synapse organizer we are trying to develop a methodology by which we can soon record cell-type-specific recordings. For the development of prob/sharp microelectrode-based electrophysiology, especially for specific cell recording, we need to develop engineered synapse organizers. It will be helpful to attach specific proteins attached with prob/sharp microelectrode and then we can create a bridge for the specific cell recordings. Synapse organizer properties are helpful to make those types of recordings, but for that, I must use a genetically engineered organizer. For making genetically engineered synapse organizers I used their extracellular part for genetic engineering. Both post and pre-synaptic organizers were used to make genetically engineered synapse organizers. Used various types of protein which are orthogonally attached with the specific protein.

There are several numbers of pre-synaptic organizing proteins. Neurexins (Nrxs) work as presynaptic organizers and are well-known directors of synapse effects thus playing a vital role in gathering and rebuilding structure through performing with many presynaptic and postsynaptic molecules or ligands. Neuroligins (NLs) are the most especially known Nrx partners. Five NL genes are present in human beings, and they are called NL1, NL2, NL3, NL4, and NL5. Through alternative splicing, NL and Nrx make their bonding, and both are controlled by their gene. Nrx and NL furnish trans-synaptic affinity through Ca^{2+}-dependent interlinkages of their substitute spliced outer cellular domains. The principal outer cellular domain of NLs expresses analogous with acetylcholinesterase (AChE) but misses cholinesterase interest and they mediate linking to Nrxs. Over exhibition of AChE, losses amounts of β-Nrxs in vivo and cell culture then lessen the evolution of glutamatergic synapses in cell culture, which indicates that may be crosstalk within two or more proteins.

In the orthogonal test, I found GFP-nanobody containing Nrx bounds with GFP and venus protein. mCherry-nanobody contains Nrx bounds with mCherry protein. mCherry contains Nrx bounds with mCherry-nanobody protein. Spot and BC2 contain Nrx bound with BC2-nanobody and spot-nanobody proteins respectively. GFP-containing Nrx showed their existence extracellularly in the dark fluorescence view and can bind with GFP-nanobody. I did a cell-microbead interaction experiment. In the cell-microbeads interaction experiment, I confirmed with a presynaptic marker called an anti-synaptophysin antibody. GFPnull/YFP containing engineered NrxΔ1 bound with GFP-nanobody microbead and confirmed by postsynaptic Anti-FLAG and Rab-3 markers. It was not easy to develop a workable engineered synapse organizer from the natural one. I considered the working principle of the synapse organizer and then thought to apply it in prob/sharp microelectrode-based electrophysiology for the recording of a specific cell. Also, we need to consider the microelectrode array with a specific medium and voltage for the prob/sharp microelectrode-based electrophysiological specific cell recording. In the future, a specific probe/sharp microelectrode with orthogonal tested protein will be developed for the desired development of the prob/sharp microelectrode-based electrophysiological technique to initiate specific cell recording. I succeeded in the development of engineered synapse organizers. In conclusion, it can be said that my engineered synaptic organizers responded positively.

Keywords: Synapse organizer, neurexin, neuroligin, synapse, electrophysiology.