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学位の種類	博士（マテリアルサイエンス）		
学位記番号	博材第 569 号		
学位授与年月日	令和 5 年 6 月 23 日		
論文題目	Generation of Orthogonal Engineered Synapse Organizers Toward the Development of Electrophysiological Technique with Target Recognition Capability		
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論文の内容の要旨

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 amount 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.

論文審査の結果の要旨

神経細胞（ニューロン）のネットワークにおける情報の符号化・保持・処理に関する基本原理は明らかではない。実験と理論の両輪の発展が重要であるが、実験研究における主要課題の一つとして、既存の細胞電気活動の実時間計測法が未成熟な点が挙げられる。

本論文は、将来的に上記の課題に寄与することを目的に、生体内でニューロンが精緻に配線される際に重要な役割を果たすことで知られるシナプスオーガナイザーと呼ばれる分子群を基礎として、生体に備わる内在性因子とは交差反応を起こさない、人工改変オーガナイザーを設計することが可能か否かを検討したものである。具体的には、プレシナプスに存在するニューレキシン 1βと呼ばれるオーガナイザーの細胞外 LNS の除去が、シナプス誘導活性に及ぼす影響を解析した。LNS ドメインはポストシナプスに存在するオーガナイザーの一種であるニューロリジンとの結合界面を形成することが知られている。LNS ドメインの除去は、実際にニューロリジンとの結合を阻害したが、依然として、細胞外エピトープを介した分子集積によりプレシナプスを誘導することが出来ることを見出した。すなわち、ニューレキシン 1βにおいて、標的分子の認識という入力を受ける部位と、シナプス誘導という機能出力する部位は、互いに独立した領域が担っていることを見出した。この事実は、LNS ドメインを任意のエピトープに置き換えることで人工のシナプスオーガナイザーとその活性化因子のペアを設計できることを意味していた。実際に、

LNS ドメインを蛍光タンパク質で置換した人工改変ニューレキシンを作製することにより、蛍光タンパク質に対する単一ドメイン抗体（nanobody）によりプレシナプスを誘導することを実験的に実証した。さらに同様の手段をニューロリジン側に適用することにより、ポストシナプスを誘導する人工改変オーガナイザーも設計できる可能性を見出した。

本論文の成果により、将来的に、ニューロンの配線を遺伝子工学的に精緻に編集したり、また、微小電極技術と融合することによる標的特異性を示す微小電極の開発など、複数の興味深い展望が開けた。これは大きな学術的な貢献と判断できる。よって博士（マテリアルサイエンス）の学位論文として十分価値あるものと認めた。