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学位論文題目 Microglial direct contacts with synapses enhance synaptic
activities in awake mice

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博士論文の要旨

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論文題目 **Microglial direct contacts with synapses enhance synaptic activities in awake mice**

Microglia are the primary immune cells in the central nervous system (CNS). They constitute 10 – 15% of all cells in the CNS. Microglia activate in injuries and diseases, changing their morphology, and having an impact on neurodegenerative disorders. In addition to their role in disease, recent studies showed that microglia are highly motile cells in the healthy brain, extending and retracting their processes. Moreover, they make direct contacts with synapses to monitor synaptic activity in an activity dependent manner. Recent evidence using embryonic zebrafish optic tectum showed that microglia also have direct contact on neuronal cell body to modify neuronal activity. In motor learning, microglia have an important role in promoting learning-related synapse formation through BDNF signaling. On the other hand, in the lever-push motor learning, the relationship between neuronal activity and individual movements becomes more consistent with motor learning.

These results lead my hypothesis that microglia could modify synaptic activity by their direct contacts to change local network activity during motor learning task. Therefore, in this study I aimed to investigate whether microglia play a substantial role of motor learning task or not, whether microglia can change populational neuronal

activities in primary motor cortex which is known as the most prominent motor output area, and whether microglia could change synaptic activity by their direct contacts or not.

In motor learning task, mice are trained to pull the lever to receive water as a reward. To investigate whether microglia can promote motor learning in this lever pull task experiment, double transgenic mice depleted microglia partially were used. In this microglia depletion mouse experiment, Iba1-tetracycline transactivator (Iba1-tTA) mouse and tetracycline operator-diphtheria toxin A (tetO-DTA) mouse were crossed. Using tetracycline-controllable gene expression system, Diphtheria toxin A (DTA) is expressed in mice which tTA is expressed if doxycycline (DOX) is withdrawn as a feed of these mice. I also used activated microglia mice which are injected Lipopolysaccharide (LPS) into peritoneal cavity to investigate whether this is only a property of resting microglia or not. I showed that success rate in control mice during motor learning increased more than that in microglia depleted mice and in LPS injected mice.

Next, I investigated whether neuronal activity in primary motor cortex are changed during motor learning task through microglial role, I measured Ca^{2+} transient as a neuronal activity using *in vivo* two-photon microscope imaging. I showed that microglia depletion mice (Dox off mice) and microglia activated mice (LPS injection mice) showed less synchronous activity in response to lever-pull movement compared with

control mice during motor learning. Moreover, I investigated populational neuronal activities in primary motor cortex during motor learning. I showed that the activity of layer 2/3 pyramidal neurons of the primary motor cortex in control mice showed stronger negative correlation between the correlation co-efficiency of the paired cells and the distance of the paired cells compared with that of Dox off mice and LPS injection mice during motor learning. It is demonstrated that activated microglia and microglia depletion suppress the synchronicity of local neuronal network activity. These results suggested that microglia play an important role in synchronizing network activity triggered by a motor learning task.

Finally, I confirmed whether microglial contact on synapse can induce a change of synaptic activity. I used AAV constructs into layer 5 primary motor cortex in Iba1-EGFP mice. This enabled me to visualize both microglia and synaptic activity in neuron simultaneously. In this experimental design, I showed local calcium responses in the postsynaptic spines increased significantly when microglial processes have contacts with spines. Activation of microglia by applying LPS inhibit spine activation with microglia contacts.

In conclusion, my result is that microglia enhance synaptic activity with their contact and this promotes the synchronized network activity during motor learning. When microglia contact on synapse, it would enhance single neuronal activity which induce higher synchronicity in the functional subnetworks.

In discussion, there are two possibilities of connection between the synchronicity of neuronal network activity during motor learning task and enhancement of synaptic activity through microglial direct contact with synapses. One is that microglia have multiple processes and could simultaneously contact on synapses of various neurons within their territory to promote the synchronization of local network activity during motor learning task. The other possibility is to progress the synchronization of neuronal activities inside the subnetwork related to the motor learning. This is the new mechanisms of microglia have an important role of promoting the synchronized network activity by direct contact of synapses.

Thus, in the physiological state, microglia have an important function to promote synchronizing network activity and can be envisaged as key neuro modulators in the brain.

Results of the doctoral thesis screening

博士論文審査結果

Name in Full
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Title
論文題目 Microglial direct contacts with synapses enhance synaptic activities in awake mice

ミクログリアは中枢神経系グリア細胞の一つで、中枢神経における唯一の免疫細胞である。これまでミクログリアの病態生理に関わる研究は多く行われてきたが、ミクログリアの生理的役割に関しては、十分に解析されていなかった。

申請者、穂吉亮平氏は、ミクログリアの生理機能を明らかにするために、(1) マウスのレバー引き運動学習におけるミクログリアの役割、(2) 運動学習中に運動野で観察される神経細胞集団の同期発火に対するミクログリアの役割、および (3) ミクログリアとシナプスの直接の接触がシナプス活動に及ぼす影響、を検討した。実験手法としては、(a) ジフテリア毒素 A をミクログリアに発現させることによるミクログリアの時期特異的除去、あるいは (b) リポ多糖 (LPS) の投与によるミクログリアの活性化を行い、覚醒マウス脳の *in vivo* イメージングを行った。

まず、申請者はミクログリアの除去もしくは活性化により、マウスのレバー引き運動学習の成功率が有意に低下することを見出した。つまり、生理的環境下のミクログリアは、マウスのレバー引き運動学習に必要であることを見出した。次に、申請者はレバー引き運動学習中の運動野 2/3 層の神経細胞集団の発火活動を G-CAMP を用いた 2 光子カルシウムイメージングにより可視化した。その結果、野生型マウス脳では、特定の神経細胞集団が同期発火することを見出した。一方、ミクログリアを除去、あるいは活性化すると、この局所神経回路の同期発火は有意に阻害された。さらに、申請者は、ミクログリアとシナプスの接触過程を覚醒マウス脳で可視化すると共に、接触過程における樹状突起スパインでのカルシウム動態を測定、解析した。興味深いことに、ミクログリアとシナプスが接触すると、そのシナプスでは特異的にカルシウム上昇が認められ、この上昇は LPS 投与によるミクログリアの活性化により阻害された。以上の結果より、申請者は生理的環境下のミクログリアは、シナプス活動を直接制御することで、局所神経回路の同期発火を促進し、運動学習に重要な役割を果たすことを明らかにした。

今回のミクログリアの生理機能解明に関する研究は、これまで病態生理に関する研究が主流であったミクログリア研究に、大きなインパクトを与え、当該分野の発展に大きく貢献するものと考えられる。とりわけ、申請者が樹立した「覚醒マウス脳におけるグリア細胞と神経細胞の接触過程の可視化技術」は、今後の脳科学分野に大きな波及効果をもたらすことが期待される。既に本研究成果の一部は、筆頭著者として英文原著論文として発表済みである。以上のことから、本論文は、学位論文として十分な内容を有すると審査委員会において全会一致で判定された。