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学 位 論 文 題 目	マウス大脳皮質運動野における領野間結合と運動方向選択性の解析
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(別紙様式 2)
(Separate Form 2)

論文の要旨
Summary (Abstract) of doctoral thesis contents

Coordinated activities of motor cortex network are thought to be essential for elaborate movements. When animals perform voluntary movement, neurons in many brain areas change their activities. Voluntary movements have various parameters such as direction, force, and speed. Neuronal activities related to such parameters are detected especially in motor cortical areas. These activities have been studied mainly with electrophysiological methods using monkeys. In those experiments, however, it is difficult to identify input/output, cell type, or spatial distribution of task related neurons.

The first objective of my thesis is to functionally and anatomically reveal layer-projection patterns between two cortical motor areas in the mouse. There are two separate forelimb motor areas in the rodent neocortex, which are called the rostral forelimb area (RFA) and the caudal forelimb area (CFA). To understand how neuronal activities in the RFA and the CFA are coordinated during movement, it is necessary to reveal synaptic connections between the RFA and the CFA. Therefore, I developed in vivo Channelrhodopsin-2 (ChR2)-based photostimulation mapping with electrical recording of neurons (optogenetic tracing) in either ChR2 transgenic mice, where the layer 5b (L5b) neurons strongly express ChR2, or in mice where both the upper layers and L5b were transfected with an adeno-associated virus (AAV) that encoded ChR2-YFP (Yellow Fluorescent Protein) (AAV-ChR2 mice). First, I confirmed that spiking activity was rapidly induced near the photostimulation points due to direct photostimulation of nearby neurons in both ChR2 transgenic mice and AAV-ChR2 mice. Next, I performed optogenetic tracings. Photostimulation of the RFA in ChR2 transgenic mice reproducibly induced spiking activity in layer 5 (L5) in the CFA. By contrast, stimulation of the CFA did not induce any detectable spiking in L5 in the RFA of ChR2 transgenic mice. Spiking activity was not detected in layer 2/3 (L2/3) in either the RFA or the CFA after photostimulation of the other areas. Photostimulation of either the RFA or the CFA in the AAV-ChR2 mice induced spiking in L5 in the other areas. By contrast, spiking activity was not detected in L2/3 in either the RFA or the CFA when the other areas were photostimulated in AAV-ChR2 mice, although both spontaneous and evoked activities were detected in L2/3 near the photosimulation sites. These results indicate that neurons in the upper layers, but not L5b, of the CFA induce strong postsynaptic responses in L5 of the RFA, and that RFA neurons in L5b induce strong postsynaptic responses in L5 of the CFA. All anatomical results obtained with anterograde or retrograde fluorescent tracers were consistent with those obtained with the optogenetic tracings. I conclude that the neuronal activity that occurs in the RFA and the CFA during movement is generated through these asymmetric reciprocal connections.

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The second objective of my thesis is to develop a method to examine motor direction selectivity of L2/3 excitatory neurons in the CFA with in vivo two-photon calcium imaging. To do so, I developed a novel lever-pull/push task for head-restrained mice. In the first stage of learning, I trained head-restrained mice to perform a voluntary (self-initiated) lever-pull movement with their right forelimb to get a drop of water. After mice acquired lever-pull movements, I trained the mice to also perform lever-push movements in the same session. This second stage of learning consisted of two blocks: a pull-movement block and a push-movement block. The mice had to pull (push) the lever to get the reward in the pull (push) -movement block. The block was switched after 30 successes of desired movements. The push/pull switch was repeated several times in a session. The performance of lever movements improved during six training sessions. While the mice performed the lever-pull/push task, I conducted two-photon calcium imaging of L2/3 neurons in the left CFA where a genetically-encoded calcium indicator was expressed. I detected the lever-pull- and/or lever-push-related activity in many neurons and estimated the motor direction selectivity in individual neurons. The data suggest that L2/3 excitatory neurons with different motor direction selectivity may be spatially separated within the CFA.

These results demonstrate that it is possible to identify the movement direction selectivity of neurons in the mouse motor cortex with two-photon imaging. Extending my study will reveal input/output, cell types, layers, and spatial distribution of neurons with the movement direction selectivity and how these neuronal activities emerge during learning. In particular, it is important to clarify how the motor direction selectivity is generated through the asymmetric reciprocal connections between the RFA and the CFA, which I revealed in the first part of my thesis. My results help to understand how the coordination between the cortical areas executes appropriate movements.

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博士論文審査結果の要旨

Summary of the results of the doctoral thesis screening

申請者は、運動発現の大脳神経回路機構に興味を持ち、マウス大脳運動野の研究を行った。まず光遺伝学と電気記録法を個体マウスに応用した optogenetic tracing 法を開発し、二つの運動野領域、Rostral Forelimb Area (RFA) と Caudal Forelimb Area (CFA)のシナプス結合様式を層レベルで解明した。次に、マウスに頭部固定状態で前肢を用いてレバー押し引きを学習させる実験系を構築し、その課題を実行しているマウスの CFA で２光子多細胞カルシウムイメージングを行い、イメージングされた細胞活動の運動方向選択性の指標を算出することを可能とした。

チャネルロドプシン 2（ChR2）が大脳皮質 5 層に強く発現しているトランスジェニックマウス(ChR2 マウス)の脳表に青色レーザーを照射することで、前肢運動を誘発するふたつの領域、RFA と CFA を同定した。次に、ChR2 マウス大脳皮質で光刺激を行いながら RFA または CFA で活動電位応答を記録する optogenetic tracing 法を開発し、これを行った結果、RFA の 5 層から CFA5 層の細胞に強い機能的シナプス入力が存在することを見出した。次にアデノ随伴ウイルス（AAV）を用いて ChR2 をいずれかの領域の全層に発現させてから optogenetic tracing 法を行うことで、CFA2/3 層から RFA5 層へ強い機能的シナプス入力があることを見出した。逆行性蛍光トレーサーと順行性蛍光トレーサーを RFA または CFA に注入し、各領域へ軸索投射する細胞の存在する層と軸索投射する層を定量化し、その結果が optogenetic tracing の結果と一致するものであることを実証した。得られた RFA と CFA 間の層非対称的投射様式は、RFA が CFA より高次な領域であり、霊長類の高次運動野と相同な領域であることを示唆した。

次に申請者は、マウスが頭部固定状態で右前肢を用いてレバーを２方向に動かす課題を開発した。まず頭部固定マウスの前肢レバー引き課題装置を改良して、レバーを押し方向または引き方向のみに駆動できる制御装置を作製した。次に、頭部固定マウスにレバー引き運動をまず学習させたのち、レバー押し運動と引き運動を同一セッション内で交互に行わせる課題に切り替えることで、引き運動に加えて押し運動も学習させることを可能とした。次に、この課題を実行中のマウスにおいて、左脳 CFA2/3 層でカルシウム感受性蛍光タンパク質を用いた２光子多細胞カルシウムイメージングを行った。カルシウム感受性蛍光タンパク質は AAV を用いて CFA に発現させた。レバー押し時と引き時の蛍光変化強度の違いから、個々の細胞の活動の運動方向選択性を算出することを可能とした。押し運動により強く反応する細胞群と、引き運動により強く反応する細胞群がやや分離して分布するイメージング領域を見出した。

以上の申請者の研究は、マウスを含む哺乳類の運動野間の機能的・構造的結合の理解に大きく貢献すること、異なる運動がどのように運動野で学習を通じて獲得され多細胞活動として表現されるかを解明するうえでの重要な研究であることが認められ、博士論文に値するものと判定した。