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学 位 論 文 題 目　Development of the connections between fast-spiking interneurons and pyramidal neurons in mouse visual cortex

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博士論文の要旨

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論文題目　Development of the connections between fast-spiking interneurons and pyramidal neurons in mouse visual cortex

Fast-spiking inhibitory interneurons (FS neurons), a major population of inhibitory neurons in the neocortex, have important roles in cortical function. It was previously reported that FS neurons preferentially form reciprocal connections with adjacent pyramidal neurons, sending inputs to the FS neurons in the visual cortex and prefrontal cortex. The reciprocal pairs of FS and pyramidal neurons often make strong synaptic connections with each other. These reciprocal connections are considered to contribute to controlling excitatory-inhibitory balance and inducing high-frequency oscillation in the neocortex. Thus, the development of reciprocity in FS neurons and pyramidal neurons seems important for the maturation of cortical functions. However, the developmental process and mechanisms remain unclear.

This study investigated the development of reciprocal connections between FS neurons and pyramidal neurons in mouse primary visual cortex. Mice were used at three developmental stages: before eye-opening (postnatal day [P]10-13), just after (P14-16), and 1 week after eye-opening (P21-26). Dual whole-cell recordings were conducted from FS neurons and pyramidal neurons in the visual cortical slices and synaptic connections between these neurons were examined. The proportion of pairs reciprocally connected increased just after eye opening. This was due to the increase in the proportion of inhibitory connections. The amplitude of unitary inhibitory postsynaptic currents (IPSCs), which was recorded from pyramidal neurons in response to an action potential in FS neurons, significantly increased from P10-13 to P14-16 and thereafter remained unchanged. Comparing the IPSC amplitude between one-way and reciprocally connected pairs, there was no significant difference at P10-13. The amplitude in

reciprocal pairs was larger than in one-way inhibitory connected pairs at P14-16, and these reciprocity-dependent strong IPSCs were maintained up to P21-26. The analysis of coefficient of variation of unitary IPSCs and paired-pulse ratio suggested that the large IPSCs in reciprocal pairs were due to an increase in the number of functional inhibitory synapses. The amplitude of unitary excitatory postsynaptic currents (EPSCs) also increased from P10-13 to P14-16, but subsequently decreased at P21-26. No significant difference was found in the EPSC amplitude between reciprocally and excitatory one-way connected pairs at P10-13. At P14-16 and P21-26, the EPSC amplitude in reciprocal pairs was considerably larger than in one-way excitatory connected pairs. These results demonstrated that the unitary IPSCs and EPSCs in reciprocal pairs were selectively strengthened after eye-opening, although this strengthening did not occur in one-way connected pairs.

Mechanisms for the establishment of high reciprocity and reciprocity-dependent potentiation of synaptic connections were explored. First, I examined the effects of visual deprivation using dark reared mice from birth until the time just before slice preparation at P21-26. Dark rearing did not affect the proportion of connectivity in FS and pyramidal neuron pairs. However, the potentiation of IPSCs in reciprocal pairs was impaired by dark rearing. The potentiation of EPSCs in reciprocal pairs occurred in dark reared mice, similar to age-matched normal mice.

Next, I examined the effect of the deletion of N-methyl-D-aspartate receptors (NMDARs) which is a key molecule for synaptic plasticity. To this end, a GluN1-flox mouse line was used to perform conditional knock-out (cKO) of GluN1, the essential subunit of NMDARs. Cre recombinase was induced by three different promoters: CMV for the ubiquitous KO, CaMKII for the pyramidal neuron-specific KO, and mDlx for the inhibitory neuron-specific KO. None of these cKO of GluN1 affected the proportion of synaptic connections in FS and pyramidal neuron pairs. However, the specific potentiation of IPSCs in reciprocal pairs was inhibited by CMV and mDlx, but not

CaMKII dependent KO of GluN1, demonstrating that NMDARs on inhibitory cells were important to establish reciprocity-dependent potentiation of IPSCs. These three types of GluN1 cKO did not affect the potentiation of EPSCs in reciprocally connected pairs, indicating that the potentiation did not depend on NMDARs.

The current study showed that both IPSCs and EPSCs in reciprocally connected FS and pyramidal neuron pairs were specifically potentiated soon after eye opening. Visual experience and NMDARs on inhibitory cells were necessary for the reciprocity-dependent potentiation of IPSCs, but not for the potentiation of EPSCs. These results suggest that the strength of inhibitory and excitatory connections, depending on the connectivity of FS neurons and pyramidal neurons, is modified by different mechanisms during development. This study provides new insight into the developmental mechanisms of connection specificity between FS neurons and pyramidal neurons in the visual cortex.

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博士論文審査結果

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Title
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抑制性細胞サブタイプのうち、Fast-spikingの発火様式をもつ神経細胞（FS細胞）は大脳皮質の主要な抑制性細胞であり、大脳の機能発現に重要な役割を担う。FS細胞は近傍に存在する興奮性錐体細胞と高い確率で双方向性結合すること、この双方向性結合ペアは、一方向性に結合するペアに比べて強いシナプス結合を形成することが報告されている。双方向性結合の形成過程を明らかにすることは、その機能的役割を理解する上で重要と考えられる。本研究では、マウス大脳皮質一次視覚野を対象にFS細胞－錐体細胞間の双方向性結合の発達過程とそのメカニズムについて解析が行われた。

先ず、通常の発達過程における結合変化を見るために、開眼前、開眼直後、生後3週齢のそれぞれのマウス一次視覚野から切片標本を作製し、2/3層のFS細胞と錐体細胞のペアからホールセル記録を行い、シナプス結合を調べた。双方向性に結合するペアは開眼直後に増加し、その後は変化しなかった。抑制性シナプス後電流（IPSC）の振幅を双方向性結合ペアと一方向性に抑制性結合するペアで比較したところ、開眼前には両ペアから記録されたIPSCの振幅に差がなかったが、開眼直後から双方向性結合ペアのIPSCが一方向性の結合ペアに比べて有意に大きくなった。FS細胞－錐体細胞ペアの興奮性シナプス後電流(EPSC)においても、開眼後から双方向性結合特異的な振幅の増大が見られた。

次に、双方向性結合形成は生後の視覚経験に依存するかを調べるために、出生直後から暗室飼育を行い、視覚体験を遮断した状態で3週齢まで飼育したマウスを用いて解析を行った。暗室飼育してもコントロールと同様に双方向性結合するペアが多くみられたが、双方向性と一方向性結合ペア間のIPSC振幅の差は小さくなった。一方、EPSCの振幅は暗室飼育の影響を受けなかった。

最後に、IPSCの可塑性に重要なNMDA型受容体の効果を細胞種特異的に欠損させることにより調べた。その結果、錐体細胞特異的なNMDA受容体欠損では、正常発達と同様にIPSCの増大が一方向性ペアよりも双方向性結合ペアにおいて顕著であったが、FS細胞と錐体細胞を含むすべての細胞での欠損あるいは抑制性細胞特異的欠損では双方向性結合ペアにおけるその優位性が消失した。したがって、FS細胞のNMDA受容体が抑制性結合発達に重要であると考えられる。一方、双方向性結合特異的なEPSCの増大はNMDA受容体欠損の影響を受けなかった。以上の結果は、視覚野FS細胞—錐体細胞ペアの抑制性結合と興奮性結合の発達は異なるメカニズムにより制御されることを示唆する。

成熟動物では、大脳皮質の錐体細胞が興奮性出力結合するFS細胞から、多くの場合抑制のシナプス結合入力を受ける。この相互結合がFS細胞の多様な機能の発現に関与すると考えられている。出願者は、この特異的な結合の形成や、その抑制・興奮強度の発達変

化だけでなく、それらの視覚入力や NMDA 受容体への依存性を初めて明らかにした。これらの結果は、視覚野回路のシナプス形成機構や FS 細胞の機能の理解に大いに貢献することから、審査委員会は本論文が学位の授与に値すると判断した。