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学 位 論 文 題 目　Characterization of inhibitory connections originating from CCK/CB1-positive interneurons in mouse primary visual cortex

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博士論文の要旨

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論文題目　Characterization of inhibitory connections originating from CCK/CB1-positive interneurons in mouse primary visual cortex

In the cerebral cortex, there are various subtypes of inhibitory interneurons present. Perisomatic inhibition is mediated by two classes of interneurons that express either the neuropeptide cholecystokinin (CCK) or the calcium-binding protein parvalbumin (PV). Inhibitory synaptic connections originating from PV interneurons are strengthened after eye-opening during postnatal development in the primary visual cortex (V1), leading to the opening of critical periods of experience-dependent plasticity. It has been reported that CCK-positive interneurons express cannabinoid receptor type 1 (CB1), which plays an important role in the development and plasticity of V1. However, functional synaptic properties due to the CCK/CB1-positive interneuron subtype in V1 and their developmental process remain unclear.

In this thesis, the proportion of interneurons expressing CCK and CB1 in each layer of mouse V1 were first examined by immunostaining for proCCK, a precursor of CCK, and CB1 using VGAT-Venus transgenic mice that had all interneurons labeled by Venus. The percentage of proCCK/CB1 double-positive cells were less than 5% in all layers at two different ages (postnatal 12–13 days, [P12–13] and P21). Additionally, some percentage of interneurons were proCCK-negative but CB1-positive P12–13, while these interneurons were almost undetectable at P21. These immunostaining results demonstrated that the proportion of CCK/CB1-positive interneurons was quite small in V1.

Electrophysiological analysis targeting CCK/CB1-positive interneurons in V1 appears difficult, owing to the small number of this interneuron subtype. Thus, CCK-IRES-Cre; Dlx5/6-Flpe; RCE-dual mice were used, which were expected to express

green fluorescent protein (GFP) only in CCK-positive interneurons. Immunostaining for interneuron subtype marker proteins was conducted to confirm the subtypes of the GFP-positive cells found in these triple transgenic mice. At P21-24, cells expressing either proCCK or CB1 accounted for half of the total GFP-positive cell; however, PV, somatostatin, and neuropeptide Y (NPY) -positive cells were also present in a non-negligible proportion. None of the somatostatin-, PV-, or NPY-positive cells expressed CB1, suggesting that CCK/CB1-positive cells can be separated from other interneuron subtypes by CB1 immunostaining. The percentage of CCK/CB1 double-positive cells in the GFP-positive cells was much higher in the triple transgenic mice than in the VGAT-Venus transgenic mice. Therefore, to analyze functional synapses originating from CCK/CB1-positive interneurons in V1, GFP-positive cells in the triple transgenic mice were recorded using whole-cell patch-clamp techniques, and the recorded interneuron subtype was confirmed using post hoc immunostaining for CB1.

To investigate developmental changes in inhibitory postsynaptic currents (IPSCs) from CCK/CB1-positive interneurons to pyramidal cells, dual whole-cell patch-clamp recordings were conducted from these cells residing in layer 2/3 of V1 slices prepared from the mice at three developmental stages: P10–13, before eye-opening; P15–18, just after eye-opening and P21–25; during critical periods of visual response plasticity. The majority of recorded GFP-positive neurons demonstrated non-fast spiking (non-FS) firing patterns at all ages. Most of the non-FS cells were CB1-positive and some were somatostatin-positive. All GFP-positive cells with FS firing patterns were CB1-negative. Inhibitory connections from CCK/CB1-positive interneurons were detected in 41.4% of the recorded pairs at P11–13. The percentage decreased during development, and only 12.9% of the pairs were connected with inhibitory synapses at P21–25. In some experiments, the recorded cells were immunostained for proCCK in addition to CB1. The connection probability from proCCK-negative but CB1-positive cells to pyramidal cells tended to be higher than that from proCCK/CB1-double positive

cells, suggesting that the high connection probability at P11–13 may be owing to presence of many inhibitory connections from proCCK-negative but CB1-positive cells found only at P11–13. Finally, the IPSCs from the CCK/CB1-positive interneurons were characterized. There were no significant differences in amplitude, kinetics, and the paired-pulse ratio of the IPSCs among the three age groups. At all ages, the success rate of inhibitory transmissions was quite low. In contrast, IPSCs originating from FS cells or somatostatin-positive cells to pyramidal cells had high success rates. These results suggest that inhibition of CCK/CB1-positive interneurons is unreliable irrespective of age. The regulation of inhibitory synaptic transmission from CCK/CB1-positive cells by CB1 activation was also examined. It was observed that the application of CB1 agonist significantly decreased IPSC amplitude and success rate, while CB1 antagonist tended to have opposite effects. These electrophysiological results suggested that CCK neurons were involved in perisomatic inhibition in V1 when inhibitory connections from PV neurons were immature before eye-opening.

In conclusion, a method to identify CCK/CB1-positive interneurons in mouse V1 by combining the use of transgenic mice with marker protein expression analysis was established. Using this method, it was detected that inhibitory connections from CCK/CB1-positive interneurons to pyramidal neurons decrease during postnatal development and that synaptic transmissions are less efficient at all ages and modified by CB1.

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博士論文審査結果

Name in Full
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Title
論文題目　Characterization of inhibitory connections originating from CCK/CB1-positive interneurons in mouse primary visual cortex

大脳皮質には様々なサブタイプの抑制性介在細胞が存在する。興奮性細胞の細胞体周辺に抑制性結合を作る介在細胞サブタイプは、コレシストキニン（CCK）を発現するCCK細胞とパルブアルブミン（PV）を発現するPV細胞の２種である。これまでに一次視覚野においてPV細胞由来の抑制性シナプス結合は、生後発達期の開眼後に強化されることが知られている。CCK陽性細胞はエンドカンナビノイド受容体（CB1）を発現しており、CB1欠損マウスでは視覚野可塑性が障害されることから、視覚野の発達に関与している可能性があるが、CCK陽性細胞由来の抑制性シナプス結合の特性や発達過程は不明であった。

出願者は、まず、すべての抑制性細胞が蛍光蛋白を発現するVGAT-Venusトランスジェニックマウスを用いて免疫染色を行い、視覚野内のCCK陽性細胞の分布を調べた。CCK陽性細胞は全抑制性細胞のうち5%足らずであり、そのほとんどがCB1を共発現していた。従って、大脳皮質のCCK陽性細胞はごく少数であり、VGAT-Venusマウスを用いてシナプス特性を電気生理学的に解析するのは困難と考えられた。そこでCCK陽性介在細胞を選択的に標識するために、①CCK-Creマウス、②抑制性細胞選択的にFlpeを発現するDlx5/6-Flpeマウス、③CreとFlpeの共存によりGFPを発現するRCE-dualマウスを交配した３重トランスジェニックマウスを用いた。このマウスの大脳皮質では約４割のGFP陽性細胞がCCK/CB1を共発現していた。このマウスのGFP陽性細胞の中にはCCK陽性細胞以外の介在細胞も多く含まれていたので、GFP陽性細胞をホールセル記録し抑制性シナプス伝達を調べた後、記録細胞をバイオサイチン染色し、免疫染色と組み合わせることで、CCK陽性細胞を同定することにした。

上述のマウスの視覚野から急性スライス標本を作製し、その2/3層にあるGFP陽性細胞と近傍の錐体細胞より同時ホールセル記録を行い、抑制性シナプス結合がみられる割合を調べた。生後11-13日齢ではCCK/CB1陽性細胞が近傍の錐体細胞との間に抑制性シナプス結合がみられる割合は約４割であったが、生後３週齢までにこの割合が約１割に低下した。結合ペアから記録した抑制性シナプス後電流（IPSC）の振幅やキネティクス、伝達の成功確率は年齢に依存した差異はみられなかった。また、CCK/CB1陽性細胞からの抑制性伝達はエンドカンナビノイドによって抑圧された。以上の結果から、開眼前の未熟な視覚野ではCCK/CB1陽性細胞は視覚野内の抑制性伝達を担う主要な抑制性細胞であることが示唆された。

本研究は、トランスジェニックマウス、免疫染色、電気生理学手法を組み合わせて、マウス大脳皮質視覚野におけるCCK/CB1陽性介在細胞サブタイプの抑制性シナプス伝達の特徴を明らかにしたものである。また、このサブタイプは生後発達の初期の抑制に関与する事を示しており、視覚野の発達を理解する上で、重要な成果と考えられる。以上の結果か

ら、本研究は学位論文として十分な内容を有しているものと、審査委員会において全員一致で判断した。