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論　文　内　容　の　要　旨

氏　　名　（　河村　菜々実　）	
論文題名	Role of clustered protocadherin-γ in PV-positive neurons for the formation of cortical neuronal circuit (大脳皮質神経回路形成におけるPV陽性細胞のクラスター型プロトカドヘリンの役割)

論文内容の要旨

In the cortex, functional neural circuits are formed by specific neural connections between inhibitory and excitatory neurons. However, the molecular mechanisms for the recognition of synaptic partner have remained unclear. I focused clustered protocadherin (cPcdh) which is a diversified membrane adhesion molecule as a candidate for the recognition molecules between neurons. cPcdh consists of 58 isoforms which are divided into three gene cluster structures (α, β, and γ) in its genome and they have homophilic adhesion property. In the CNS, cPcdhs are expressed in most of neurons. Around 15 isoforms are stochastically expressed in different combinations in each cell, which gives each cell its individuality. It has been reported that the deletion of cPcdhγ in inhibitory neurons in the telencephalic region, abnormal long-lasting neural activity had been observed by whisker stimulation in the somatosensory cortex. These results suggest that cPcdhγ in inhibitory neurons could be involved in the formation of appropriate cortical neural circuits. A variety of inhibitory neurons exist in the cerebral cortex. Among them, parvalbumin expressing (PV^+) neuron occupies the 50% of inhibitory neural population and high frequently forms reciprocal connections with pyramidal neurons. It is thought to play important roles in information processing by modulation of visual response property of pyramidal cells and generation of the cortical gamma oscillation. In this study, I investigated the influence of cPcdh γ deletion in PV^+ neurons on local neural circuits in the primary visual cortex of mice by whole-cell patch clamp recording in conditional cPcdhγ deletion mice in PV^+ neuron (*PV-Cre*; *cPcdhγ*$^{fl/fl}$: KO mice). The deletion of cPcdhγ in PV^+ neuron did not affect the membrane properties of PV^+ neuron and pyramidal neuron. To examine the connection between PV^+ neuron and pyramidal neuron, simultaneous double whole-cell recordings were performed. The connection probability between PV^+ neuron and pyramidal neurons and the strength of unitary inhibitory synaptic responses were similar between control and KO mice. I examined the connection relationship between PV^+ and multiple neighboring pyramidal neurons in control mice by multiple whole-cell recordings. As a result, I found two types of PV^+ neurons with different connectivity with pyramidal neurons: a high reciprocal connectivity to neighboring pyramidal neurons (high-reciprocity) and low-reciprocity. Surprisingly, those classification was lost in KO mice, indicating that cPcdhγ might determine the differential connectivity of each PV^+ neuron within layer 2/3. To reveal the influence of cPcdhγ KO in interlaminar connectivity, I performed excitatory input map through layer 2/3 to layer 6 by whole cell recording and laser-scan photostimulation using caged glutamate. I found that cPcdhγ-deleted PV^+ neuron receive more inputs from layer 5 and layer 6 pyramidal cells compared to control mice. These results indicate that cPcdhγ is involved in not only intralaminar connections but also interlaminar connections in the visual cortex. To investigate the relationship between expression patterns of cPcdhγ isoforms in each neuron and neural connectivity, I combined whole-cell patch clamp with single cell-RNA sequence. I found that reciprocally connected PV^+ neurons and pyramidal neurons shared same type of cPcdhγ isoforms significantly more compared to shuffled data.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、特異的な細胞接着活性をもつ多様化膜認識分子であるクラスター型プロトカドヘリン(cPcdh)のなかでも、抑制性神経細胞において重要な役割を果たしているcPcdh γに着目することで、機能的な情報処理の根底にあるシナプス結合の特異性決定メカニズムに迫っている。今回、マウス一次視覚野2/3層でのホールセルパッチクランプ記録により、パルブアルブミン陽性(PV^+)抑制性神経細胞に発現するcPcdh γが、PV^+神経細胞と近隣の複数の興奮性神経細胞との間に構成される局所神経回路の特異性を消失させることを突き止めた。これは、2細胞間におけるシナプス結合能や強度にはcPcdh γは関与していないことから、cPcdh γが特定の結合相手を認識し、特異性を生み出すことを示唆する結果である。また、他層から2/3層のPV^+神経細胞への興奮性入力の層特異性があることを今回初めて発見し、その特異性にもcPcdh γが関与することも突き止めた。本研究は、１細胞レベルでの特異的結合の鍵となるもcPcdh 分子と局所神経回路の関係に焦点を当てることで、分子レベルから局所神経回路形成の特異性を明らかにした研究であり、博士号取得に値するものであると考える。