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学位論文題目	Dendritic domain-selectivity of intracortical excitatory inputs onto layer 2/3 pyramidal neurons
論文審査委員	主　査　教授　鍋倉　淳一 教授　吉村　由美子 教授　川口　泰雄 教授　小松　由紀夫　名古屋大学

Pyramidal neurons in the cerebral cortex are principal neurons projecting their outputs to various brain areas. A salient feature of pyramidal neurons is their distinct apical and basal dendrites, which protrude from the apex and the basal of the soma. These neurons receive excitatory inputs from various cells on their numerous dendritic spines, which amount to several thousands. The elucidation of signal integration mechanisms in the neuron with numerous inputs is pivotal to the understanding of cortical information processing. Neuronal connectivity in visual cortex has been intensively studied and specific neuronal connections are considerably unraveled. For example, layer 2/3 pyramidal neurons, sending output signals to other cortex, receive various inputs including those from outside of the cortex, such as the thalamus and other cortex, as well as those from nearby cortical cells in layer 2/3, 4 and 5. It is known that inputs from the higher-order cortex target the distal part of apical dendrites. Recent studies have clearly demonstrated that specific and non-specific thalamic neurons send their outputs mainly on the basal and distal apical dendrites, respectively. However, the spatial distribution of inputs from nearby cortical neurons on dendrites is not well resolved yet, mainly due to technical difficulties. Because apical and basal dendrites in pyramidal cells are separated by the soma which integrates signals sent from synaptic sites that distributed broadly on dendrites, synaptic inputs on apical and basal dendrites may contribute differently to the signal integration at the cellular level.

The author attempted to determine whether postsynaptic responses in pyramidal neurons in the lower part of layer 2/3 (layer 3) are mediated by inputs from nearby cortical cells on apical or basal dendrites in rat visual cortex. There are no excitatory synapses on the soma and the proximal part of apical dendrites. This spatial arrangement of the excitatory synapses was utilized to resolve this issue. The author conducted dual whole-cell voltage-clamp recordings from the dendrite and the soma of the same pyramidal cell. The author placed one patch pipette on the primary apical dendrite and the other on the soma, so that there were no excitatory synapses between the two pipettes. In this condition, it is expected that excitatory postsynaptic currents (EPSCs) recorded from the dendrite would be larger than those recorded from the soma if the excitatory inputs impinge on the apical dendrites, while opposite results would be obtained if the excitatory inputs impinge on the basal dendrites.

In order to investigate this issue, the author first analyzed inward currents evoked by focal photostimulation of the apical and basal dendrites with glutamate uncaging in the presence of the Na^+ channel blocker tetrodotoxin (TTX). The relationship between the direct responses recorded by the two electrodes was consistent with the author's expectations. The author attempted to separate miniature EPSCs (mEPSCs) into those mediated by inputs to the apical and basal dendrites. In the graph plotting the amplitude of responses recoded from the dendrite against the amplitude of the responses recorded from the soma, mEPSCs were clearly separated into two groups. One group of mEPSCs was distributed along a linear regression line with a slope larger than 1, while the other group was distributed along another line with a slope smaller than 1. Direct responses evoked by the stimulation of apical and basal dendrites of the cell were exactly distributed along the former and

latter regression lines, respectively, suggesting that it is possible to determine whether individual mEPSCs originated from apical or basal dendrites based on the amplitude ratio of responses recorded simultaneously.

The author computed a frequency distribution of the log ratios of the dendritic response amplitude to the somatic response amplitude (logR), to promote this identification of the mEPSC origin. The logR distribution for mEPSCs was well fitted by a sum of two Gaussian curves, which peaked at a negative and a positive logR value, and clearly separated by a trough at around 0 between the two peaks. The direct responses evoked by apical and basal dendrites were distributed around either the positive or negative peak. This clear dichotomy supports the view that a logR larger or smaller than the value at the trough reliably indicates apical and basal dendritic origin, respectively.

This method seemed applicable to the analysis of evoked EPSCs (eEPSCs), because the frequency distribution for spontaneous EPSCs (sEPSCs) recorded in a normal solution without TTX was also fitted with a sum of two Gaussian curves exactly as the distribution for mEPSCs. This suggests that sEPSCs were mostly mediated by inputs to either apical or basal dendrites, just like the mEPSCs. The author analyzed the eEPSCs by activating cortical neurons with laser-scanning photostimulation. In most of the frequency distribution of logR for the eEPSCs, a large negative peak was clearly seen, while the positive peak was vague and small, and some of the eEPSC were located around 0. This suggests that cortical inputs target far more basal than apical dendrites. In addition, some of inputs likely target apical and basal dendrites together.

The number of eEPSCs derived from one experiment was not enough to reliably quantify the proportion of eEPSCs mediated by inputs from each layer on the basal or apical dendrites alone, or common inputs to both apical and basal dendrites. Therefore, the author normalized the frequency distribution of logR so that the negative and positive peaks were converted to -1 and +1, respectively, and then constructed a pooled distribution for all of the tested cells. The pooled distribution was fitted with a sum of two Gaussian curves with the peaks at -1 and 1 using eEPSCs with abscissa values smaller than -1 or larger than 1, because these eEPSCs are considered to be mediated by basal or apical dendrites alone. The author estimated the percentage of apical and basal eEPSCs from the fitting curve with negative and positive values, respectively, and then that of the eEPSCs due to common inputs from the difference between the distribution and the fitting curve.

The majority of inputs targeted either the basal or the apical dendrite alone, while the remaining minor inputs impinged on both dendrites. There were far more basal eEPSCs (66%) than apical eEPSCs (20%). The proportion of eEPSCs with common inputs (14%) was only slightly lower than that of the apical eEPSCs. The same type of analysis for responses evoked by the stimulation of layer 2/3, 4 and 5 showed that layer 3 pyramidal neurons received more synaptic inputs on their basal than apical dendrites from any of layer 2/3, 4 and 5. The innervations of both apical and basal dendrites were preferentially found for source neurons located near the target neuron. The strength of synaptic inputs to layer 3 pyramidal neurons was different depending on the laminar location of presynaptic neurons and the synaptic site of the dendrites. These results suggest that synaptic

inputs from different kinds of adjacent cortical neurons are integrated differently.

博士論文の審査結果の要旨

大脳新皮質の主たる興奮性細胞である錐体細胞は、その樹状突起上に広く分布する数千ものスパインで興奮性シナプス入力を受ける。樹状突起は、細胞体の上部から始まる先端樹状突起と細胞体の下部から始まる基底樹状突起からなるが、それぞれへの入力する回路については、大脳皮質の複雑な構造および技術的な困難さから殆ど未解決である。　中川氏は独自に開発した電気生理学的アプローチ法を用いて、大脳皮質 2/3 層錐体細胞の先端樹状突起と基底樹状突起に対する大脳皮質 2/3 層、４層、５層の細胞からの興奮性シナプス入力の空間的位置情報の解析を行い、全く新しい知見を見出した。詳細を以下に記載する。

ラット一次視覚野のスライス標本を作製し、単一の 2/3 層錐体細胞の細胞体と、細胞体から約 20 μm 離れた先端樹状突起の２ヶ所から同時にホールセル記録を行った。この２つの記録電極間に興奮性シナプスは存在しないので、先端樹状突起にシナプス活動が生じると細胞体よりも先端樹状突起でより大きな反応として記録され、基底樹状突起にシナプス活動が生じると先端樹状突起よりも細胞体でより大きな反応として記録されるはずである。この仮説を立証するため、各樹状突起にグルタミン酸を局所投与する実験により確認されたので、２本の電極で同時記録された興奮性シナプス後電流（EPSC）の大きさの比に基づいてどちらの樹状突起に反応が生じたかを判別した。同時記録した微小 EPSC の振幅の比の対数の分布は、ほぼ完全に分離した２つの正規分布を示し、これら２つの分布のピークは、先端あるいは基底樹状突起にグルタミン酸を局所投与した時に同時記録された反応の比の対数値にほぼ一致することが分かった。したがって、どちらか一方の樹状突起へのシナプス入力はこの分布に含まれると予想され、この分布を指標とすることでシナプス入力の位置を推定することが可能である。

この手法とケイジドグルタミン酸を用いた局所光刺激法を組み合わせて、各層の細胞からの興奮性入力が先端と基底樹状突起のどちらに送られたかを同定した。その結果、先端樹状突起および基底樹状突起のどちらか一方へのシナプス入力は全シナプス入力の80-90%を占め、残りの入力は両樹状突起に送られると推定された。したがって、個々の皮質細胞は、先端樹状突起と基底樹状突起のどちらか一方の樹状突起にのみ出力を送る傾向が強いと考えられる。樹状突起への入力の優位性は、どの層由来でも基底樹状突起に送られる割合が大きく、その割合は５層、４層、2/3 層に由来する入力の順に大きかった。2/3 層からの入力は他の２つの層からの入力と比較して、先端樹状突起に入力する割合が大きかった。したがって、大脳皮質 2/3 層錐体細胞の樹状突起への投射様式はシナプス前細胞の層により相違がある。また、2/3 層錐体細胞への入力の強度は一様ではなく、シナプス前細胞の層と、入力が終わる樹状突起部位に依存して異なっていた。以上の結果は、種類が違う細胞からの入力は、錐体細胞の樹状突起・細胞体での信号処理において異なる役割を果たすことを示唆する。

この結果は、大脳皮質における神経回路の特性および情報処理のメカニズムに全く新しい知見を与えるものであり、学位論文として十分に値するものである。