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学位論文題目	Quantification of excitatory and inhibitory synapseson GABAergic nonpyramidal cell subtypes in the rat cerebral cortex
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論文内容の要旨

The neocortex is composed of excitatory (pyramidal) and inhibitory (GABAergic nonpyramidal) neurons. Pyramidal neurons receive excitatory synaptic input from their own recurrent collaterals as well as thalamic fibers. Pyramidal neurons also receive inhibitory synaptic input from local GABAergic nonpyramidal cells. This mixture of synaptic input maintains the excitatory and inhibitory balance in the cortex. Neocortical GABAergic cells are morphologically and physiologically heterogeneous, but specific subtypes can be identified based on differential expression of specific peptides and proteins. Individual GABAergic cell subtypes tend to innervate specific surface domains of other cortical cells. Somatostatin-expressing Martinotti cells mostly innervate thin dendritic shafts and spines, whereas parvalbumin fast-spiking (FS) basket cells also make synapses on somata. Thus, cortical inhibition is differentially exerted onto specific cellular domains based on the innervation patterns of different interneuron subtypes. Therefore, to understand the mechanisms that maintain the excitatory and inhibitory activity balance in the cortex, it is necessary to reveal the specific excitatory and inhibitory input patterns onto individual GABAergic cell subtypes.

GABAergic neuron subtypes show differential dendritic spatial extension, branching patterns, and spine densities. The local input impedance influences local postsynaptic potentials induced by active synaptic conductances, and is in turn dependent on the postsynaptic dendritic geometry. Local synaptic current amplitudes are related to the postsynaptic synapse density and junctional area related to the receptor number. The total excitatory depolarization would be determined by interaction between the activated excitatory and inhibitory synapses. However, it remains to be investigated how local postsynaptic morphologies, important for the local synaptic integration and current transfer to the soma, are related to synaptic density. Furthermore, it is not known if these relationships are different between excitatory and inhibitory terminals onto the various GABAergic neuron subtypes. The cell body integrates all excitatory currents from the dendrite and generates depolarization for spike induction. The differences in excitatory and inhibitory balances would affect the firing regulation a lot.

The majority of GABAergic neurons can be identified by chemical expression of parvalbumin, calretinin and somatostatin. These chemical classes are further divided into subtypes, such as a somatostatin subpopulation expressing nitric oxide synthase (NOS). Here they have investigated the relationships between postsynaptic density of GABA-positive and GABA-negative terminals onto different GABAergic neuron subtypes.

First they confirmed that substance P receptors (SPR) were selectively expressed in NOS cells, a subpopulation of somatostatin cells (13% of somatostatin cells in layer

II/III, 20% in layer V and 25% in layer VI) by double immunofluorescence. Parvalbumin and calretinin cells were not positive for SPR. Next they labeled the somata and dendrites of 4 chemically defined nonpyramidal neuron subtypes positive for somatostatin, SPR, parvalbumin, or calretinin by pre-embedding immunohistochemistry using Ni-DAB reaction. These sections were embedded in Epon for electron microscopic observations. Some immunostained somata and dendrites were reconstructed 3-dimensionally at the light microscopic level using the Neurolucida system. Immunopositive tissues were serially sectioned in 90 nm thicknesses. To identify GABAergic terminals, they applied GABA postembedding immunohistochemistry to ultrathin sections, detected by colloidal gold particles. Synaptic boutons were quantitatively divided into two classes on the basis of gold particle densities for GABA immunohistochemistry. The particle density differences between GABA-negative and -positive terminals were similar among the materials immunostained for the above 4 chemical markers.

The labeled somata and dendrites and associated structures were reconstructed from serial electron microscopic images by a 3D reconstruction system using the software package 'Reconstruct'. From the reconstructed dendrites, they measured the length and surface area, followed by a calculation of the averaged cross-sectional area. In individual reconstructed dendritic segments, they counted GABA-positive and -negative synapses, followed by evaluation of their density per surface area.

Cell bodies of 4 chemical types were partially reconstructed, and somatic synaptic input patterns were compared between them. GABA-positive synapse densities on the soma were similar between the subtypes, but GABA-negative densities were significantly different. Parvalbumin cells had higher densities of GABA-negative synapses than did calretinin and somatostatin cells. Therefore, the proportion of GABA-positive synapses on the soma was significantly different between the 4 classes. Somatostatin somata had a higher proportion of GABA-positive synapses than did SPR and parvalbumin somata. Calretinin-positive somata had a higher proportion of GABA-positive synapses than those of parvalbumin cells. These indicate that nonpyramidal neuron subtype influences the ratio of inhibitory to excitatory somatic input.

Dendritic spines were found in somatostatin cells, but not in those of parvalbumin and calretinin cells. Although SPR cells were a subpopulation of somatostatin cells, spines were not identified in SPR dendritic segments.

The dendritic synaptic densities and cross-sectional areas were well correlated in GABA-negative synapses. Larger dendrites were lower in GABA-negative synapse density, and smaller dendrites had higher synaptic densities. The density dependency on the postsynaptic dendritic dimension was most prominent in SPR cells and least in calretinin cells. On the other hand the correlation between GABA-positive synapse densities and dendritic dimensions was weaker than that of GABA-negative synapses.

These data show that GABA synapse density is relatively constant between dendritic locations, but excitatory input density changes according to the postsynaptic dendritic dimension and location.

They next compared dendritic synaptic densities as a whole. GABA-positive synapse densities on dendrites were similar between the neuronal subtypes, but GABA-negative synaptic densities were significantly different. Calretinin dendrites had lower GABA-negative densities than did parvalbumin and SPR cells. Somatostatin dendrites were lower in GABA-negative densities than were parvalbumin-positive neurons.

These observations revealed that the GABAergic inhibitory synaptic density is similar between the subtypes, the somata and dendrites, the dendritic surface locations, or the dendritic dimensions. On the other hand, the excitatory density varies between the subtypes. It is higher in dendrites than in somata, and also higher in distal thinner dendrites.

論文の審査結果の要旨

大脳皮質には発火様式・形態・分子発現が異なる多様なGABA作働性ニューロンがあるが、その機能分担は未だによくわからないことが多い。これらのGABA作働性ニューロンのシナプス結合パターンについては、サブタイプごとに出力する標的細胞や細胞膜ドメインが異なることがよく知られている。一方GABA細胞軸索終末の後シナプス構造の解析に比べて、樹状突起への興奮・抑制性入力分布やそのサブタイプごとの違いは殆ど調べられていない。そこで本学位論文では、大脳皮質GABA細胞のサブタイプ特異的なシナプス入力ルールを知るために、ソマトスタチン、サブスタンスP受容体(SPR)、パルブアルブミン、カルレチニンを発現する4種類のGABA細胞サブタイプの細胞体・樹状突起を包埋前免疫組織化学で標識し、電子顕微鏡観察用に連続超薄切片を作成した。さらに興奮性終末と抑制性終末を区別するために超薄切片上での包埋後免疫組織化学によるGABAの標識を行い、細胞体・樹状突起とその上の興奮・抑制性シナプス入力を三次元的に再構築し、細胞体・樹状突起の形態とシナプス分布を定量的に計測した。

計測を行った4種類のGABA細胞サブタイプは合計でGABA作働性ニューロンのうち約90%を占めていた。これらの細胞体での抑制性シナプスの単位表面積当たりの密度は4つのサブタイプ間で大きな差がみられなかった。これに対して、興奮性シナプスの単位表面積当たりの密度はサブタイプ間で大きく異なっており、パルブアルブミン細胞やソマトスタチン細胞のサブグループであるSPR細胞がカルレチニン細胞より有意に高かった。

4種類のGABA作働性ニューロンのうち、樹状突起スパインはソマトスタチン細胞だけで見られ、SPR細胞、パルブアルブミン細胞、カルレチニン細胞では殆どみられなかった。興奮性シナプスの単位表面積当たりの密度はどのサブタイプにおいても樹状突起局所の大きさに依存しており、太いものほど興奮性シナプスの密度は低くなっていた。このシナプス後部の樹状突起の太さに対する興奮性シナプス密度の依存性は、SPR細胞で最も大きく、カルレチニン細胞で小さかった。樹状突起における抑制性シナプスの密度は細胞体の場合と同様、サブタイプ間で大きな違いが見られなかったのに対して、興奮性シナプスの単位表面積当たりの密度はSPR細胞やパルブアルブミン細胞で高く、カルレチニン細胞で低かった。

これらのことから、1）GABA陽性シナプス（抑制性入力）の密度はサブタイプによってあまり差が無く、細胞体上と樹状突起上、樹状突起上での位置、樹状突起の太さなどの違いに関わらず良く似ていること、2）GABA陰性シナプス（興奮性入力）の密度はサブタイプによって異なり、細胞体より樹状突起で、またより径が細い遠位樹状突起で密度が高いことが明らかになった。

以上の結果は、それぞれのサブタイプについて統計的解析を可能とするデータ量に基づいて結論されており、数多くの超薄連続切片の立体再構築を必要とする、大変な労力と時間を要する仕事であることは明らかである。出願者は忍耐強くこのような実験を重ねており、その結果に基づいた定量的な解析の信頼度は高い。本研究の成果は、大脳皮質作働性ニューロンにおいて、抑制性入力がサブタイプや細胞膜ドメインの違いによらず比較的均等に分布しているのに対し、興奮性入力の収束様式がサブタイプごと、また樹状突起の細胞体からの距離に応じて異なることを初めて定量的に明らかにしたもので、皮質内GABA作働性回路の機能分化の解明に貢献するものである。よって、本研究が学位論文としてふさわしいものであることに、審査委員全員の意見が一致した。