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論文内容の要旨

Synaptic connection patterns between pyramidal cell subtypes in layer V of rat frontal cortex

Pyramidal cells in the cortex are heterogeneous in their extracortical projection sites. Excitatory pyramidal cells are recurrently connected with eachother. The connection specificity among diverse pyramidal cells is crucial for understanding the local circuit organization. However, the local synaptic connectivity and excitatory interactions between the same ordifferent types of pyramidal neurons remain to be investigated. In the frontal cortex, corticostriatal pyramidal cells in layer V are composed of two classes based on their axonal projection patterns (1) those projecting to the pons (corticopontine cell, CPn cell), often with collaterals to the striatum, and (2) those projecting to both sides of the striatum, but not to the pons (crossed corticostriatal cell, CCS cell). The intracortical interaction between different channels of the cortex and basal ganglia loop is critical for their functions. Here she shows that subpopulations of corticostriatal neurons in the frontal cortex are selectively connected with each other based on their subcortical targets.

At first, she used two different fluorescent tracers injected into their projection target areas to confirm that CCS and CPn cells were distinct cell types. Double-labeled cells were never observed, indicating that two types belonged to completely separate neuronal populations. They were morphologically differentiated especially in regard to their apical tufts. CPn cells had larger tuft areas, longer length of layer I dendrites, and more branch points in layer I than did CCS cells

To reveal the synaptic connectivity between two pyramidal cell types, she recorded from pairs of retrogradely labeled corticostriatal neurons. CCS cells had reciprocal synaptic connections with each other and also provided synaptic inputs to CPn cells. However, reciprocal connections from CPn neurons to CCS cells were rarely found. The postsynaptic currents generated by presynaptic CCS neurons were quantitatively similar regardless of postsynaptic target.

To test whether there are target-specific differences in CCS synapse formation onto postsynaptic CCS or CPn cells, She reconstructed both the axons and dendrites of paired neurons. The morphological parameters were compared between presynaptic and postsynaptic cells Interconnected CCS cells often shared similar dendritic morphologies. They were quantitatively similar in morphological parameters such as the lengths of tuft branches in layer I and the mean internode intervals of basal dendrites

To measure the morphological variety of CCS cells in comparison with CPn cells, they aligned dendritic reconstructions in accordance with the somatic depth from the pia The dendritic morphologies of CCS neurons were correlated with their somatic depth from the cortical surface. Tuft dendritic lengths in layer I were heterogeneous, but were significantly shorter in neurons with their somata in the deeper areas of layer V. The internode intervals and horizontal dendritic distances of basal dendrites were longer in superficial CCS neurons than deeper CCS neurons These data suggest that while CCS cells are heterogeneous in their dendritic structures, there is a significant correlation between the size and robustness of their dendritic fields and their sublaminar position

within layer V.

The axon contact patterns on the dendrites were compared betweenpostsynaptic CCS and CPn cells. Significantly fewer contact sites were observed in CCS cells than were made onto CPn cells. CCS axons contacted apical branches more frequently in postsynaptic CPn cells than in CCS cells. She compared the mean EPSC amplitude with the contact site number and their positions from the soma along dendrites. EPSC amplitudes were more correlated with the number of contact sites rather than their spatial distribution. Further, this correlation was stronger in CCS to CCS pairs than in CCS to CPn pairs. As expected, coefficient of variation of EPSC amplitudes inversely correlated with the number of contact sites. These data suggest that the contact number reflects the number of synaptic release sites.

Given the data above, she hypothesized that CCS neurons show specificity in synapse formation onto postsynaptic dendrites. To investigate whether CCS neurons show preferences in postsynaptic targets, we compared the spatial distributions of contacts generated by presynaptic CCS axons onto postsynaptic dendrites with those of approaches (potential contact sites) in CCS to CCS or CPn pairs. This analysis suggests that CCS axons make synaptic contacts onto postsynaptic neurons with a high degree of specificity, failing to make synaptic contacts onto nontargeted neurons even though opportunities for synaptic connections exist as evidenced by numerous approach points onto both apical and basal dendrites.

These findings suggest that the two types of corticostriatal cells are hierarchically organized, and that intratelencephalic corticostriatal cells are segregated in a sublaminar fashion within layer V and often make connections with other CCS neurons sharing morphological similarities.

論文の審査結果の要旨

大脳皮質の錐体細胞は層ごとに、また同じ層内でも皮質外投射領域が異なるものがある。同一皮質領域内で投射先の異なる二つのニューロン間の皮質内結合やそれらの形態の差異についてほとんど調べられていない。ラット前頭皮質5層にある線条体へ投射する錐体細胞には二種類あることが知られている。一つは同側線条体へ軸索側枝を出し更に脳幹へ下降するもので、もう一つは両側線条体へ投射するが脳幹へは軸索を伸ばさない。この二種類の錐体細胞の形態的特徴・皮質内結合を調べることは、錐体細胞間の結合選択性を明らかにするだけでなく、大脳基底核への皮質入力の役割を理解する上でも重要である。申請者は、上記の二種類のニューロンを、橋と対側線条体に二種類の異なる逆行性色素を注入することで、corticopontine cell (CPn 細胞) と crossed corticostriatal cell (CCS 細胞) として同定し、同時にホールセル・パッチクランプ記録を行うことによってシナプス特性及び神経結合を調べた。電気生理実験終了後、記録細胞を細胞内染色しニューロルシーダで三次元再構築して形態学的解析を行い、以下のことがわかった。

1. 尖端樹状突起形態がタイプ間で異なり、CPn 細胞の方がシャフト起始部の直径が大きく、I 層におけるタフト構造が発達していた。

2. CCS 細胞の尖端・基底樹状突起の両方とも、その形態は細胞体の皮質表面からの深さに依存して変化した。

3. タイプ間のシナプス結合には方向性があり、CCS 細胞間及び、CCS から CPn 細胞への結合は約 10%で見られたのに対して、CPnから CCS 細胞への結合はほとんどみられなかった。

4. CCS 細胞からのシナプス電流の時間経過は、シナプス後錐体細胞のタイプによらなかった。軸索ブトンのコンタクト数は、CCS 細胞へのシナプス電流の大きさとよく相関し、変動係数・欠落率と逆相関していた。

5. CCS 細胞間では主に基底樹状突起にコンタクトがみられ、CCS-CPn 細胞間では尖端樹状突起にもみられた。コンタクト数は CPn細胞への方が多くみられた。

本論文で初めて線条体へ投射すると考えられる錐体細胞を二種類同定した上で皮質内結合が調べられた。その結果、これらのタイプの間には結合方向・ドメイン選択性があることがわかり、一つのタイプには更に皮質の深さに依存したサブグループがあり、同じサブグループ間で反回興奮結合が発達している可能性が高いことが分かった。

本研究はこれまで明らかでなかった大脳皮質5層における異なる種類の出力細胞同士の選択的結合様式を電気生理学的手法と形態学的手法を組み合わせて詳細に解析し、明らかにした研究であり、当該分野の発展に大いに貢献する優れた研究である。以上の理由により、申請者の論文は学位を授与するに相当する研究であると審査委員会全員一致で判断された。