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論文内容の要旨

Developmental and peripheral nerve injury-induced changes of afferent synapses in the somatosensory thalamus

Precise synaptic connections and appropriate synaptic transmission are essential for proper functions of the brain. Both of which are established via the postnatal development and can be reorganized according to new environments. Lemniscal synapses in the ventroposterior medial (VPM) thalamic nucleus are afferent synapses, which convey somatosensory information from the trigeminal nuclei. Lemniscal synapses of the adult mice are characterized by the synaptic connections that a relay neuron receives one lemniscal fiber and by the fast reliable synaptic transmission. Such characteristics are established during the postnatal development. However, the entire functional development of lemniscal synapses remains unknown. Even after lemniscal synapses become mature, functional remodeling, such as expansion and shrinking of receptive fields, in the somatosensory thalamus induced by the lesion of the peripheral sensory nerve has been described in *in vivo*. However, it is still unknown how lemniscal synapses change underlying the functional remodeling of the somatosensory thalamus. Thus, he investigated developmental changes and peripheral nerve injury-induced remodeling of lemniscal synapses using whole-cell patch-clamp technique in brain slice preparations of mice. His thesis is organized into three chapters. In the first chapter (chapter 1), he proposes three distinct stages of developmental changes at lemniscal synapses based on synaptic properties and innervation patterns of lemniscal fibers onto a relay neuron. His findings are as follows: (1) During the first stage (P0 - 6), the number of lemniscal fibers which innervate a relay neuron increases. The amplitude of total lemniscal EPSCs via both NMDA and AMPA receptors rapidly grows at the end of this stage. (2) During the second stage (P7 - 20), redundant lemniscal fibers are dramatically eliminated. As a result, a relay neuron becomes innervated by a single lemniscal fiber by P21. Silent lemniscal synapses, which are abundant during the first stage, disappear during this stage. The AMPAR/NMDAR ratio of EPSC gradually increases in a similar time course. Moreover, the decay time of AMPAR-mediated EPSCs becomes shorter during this stage. A switch of alternative splice variants of AMPA receptor subunits (from flip to flop) underlies the phenomenon. (3) During the third stage (P21~), all synaptic properties are stable, indicating that the development is already completed. In the second chapter (chapter 2), he reports the peripheral nerve cut-induced remodeling of mature lemniscal synapses. He cuts the infraorbital nerve (a part of maxillary nerve passing through infraorbital canal, which convey somatosensory information from maxillary region) of mice on P21. One week after the operation (IONC) on P21, multiple reinnervation of lemniscal fibers onto a relay neuron are observed. In addition,

the IONC operation induces silent lemniscal synapses. The IONC operation decreases the amplitude of single fiber-mediated EPSCs, but not that of total lemniscal EPSCs of a given relay neuron. Multiply innervating lemniscal fibers are classified into two distinct populations; "Strong fibers" with shorter decay time and larger amplitude of AMPAR-mediated lemniscal EPSCs and "Weak fibers" with longer decay time and smaller amplitude. A cluster analysis and innervation patterns of the two types of fibers strongly suggest that Weak fibers are newly-recruited fibers. Moreover, current-voltage relationships and pharmacological analyses indicate that the IONC operation up-regulates GluR2-containing and flip-type AMPA receptors at the postsynaptic sites of Weak fibers. In contrast, no change is observed on NMDAR-mediated EPSCs. Paired-pulse ratio and coefficient of variation of AMPAR-mediated lemniscal EPSCs are elevated at Weak fiber synapses. These results suggest that the IONC operation can induce remodeling of lemniscal synapses even after the synapses become mature. Notably, because the whisker deprivation does not multiple reinnervation of lemniscal fibers onto a relay neuron, such reinnervation are likely caused by the injury of peripheral nerve rather than the lack of whisker mediated sensory inputs. In the third chapter (chapter 3), the similarity between the developing and remodeled (by peripheral nerve lesions or such) nervous systems is discussed. The present data on lemniscal synapses exhibited the similarity; the remodeled lemniscal fibers by the IONC operation showed immature properties, including the multiple innervation and the small AMPA/NMDA ratio of EPSCs. Previous studies have reported that the remodeling of other particular synapses after nerve lesions also results in showing immature synaptic properties. Thus, the remodeling toward immature phenotypes is presumably general after the adult nervous system undergoes pathological conditions, which may suggest the recapitulation of the development. Although the normal development and the remodeling after pathological conditions would have different functional significance, they appear to share common neural mechanisms by which the nervous system actively reorganizes itself.

博士論文の審査結果の要旨

体性感覚視床における求心性シナプス（内側毛帯シナプス）が生後発達期にどのように変化するか、また成熟したシナプスが末梢神経損傷によりどのような影響を受けるのかは十分に分かっていない。竹内雄一は、マウスの急性脳スライス切片にパッチクランプ法を適応し、内側毛帯繊維を介する興奮性シナプス後電流（内側毛帯ＥＰＳＣｓ）を記録することにより、内側毛帯繊維の視床内配線変化および内側毛帯シナプスの性質変化を検討した。

生後発達の検討の結果、生後第一週は内側毛帯シナプスが強化される時期であると分かった。出生直後（Ｐ０－２）においては視床内中継細胞はそれぞれ約二本の内側毛帯繊維入力を受けた。内側毛帯繊維入力の数は生後六日目を境に増加し、約七本に達した。この時期に一致してＮＭＤＡ受容体およびＡＭＰＡ受容体を介する内側毛帯ＥＰＳＣｓの振幅が増大した。生後第二、三週は視床内神経回路の洗練化および内側毛帯シナプスの成熟化が進む時期であると分かった。内側毛帯繊維はシナプス除去を受け、生後第２１日までにそれぞれの中継細胞がただ一本の内側毛帯繊維入力を受ける成熟型の神経回路が成立した。内側毛帯ＥＰＳＣｓのＡＭＰＡ/ＮＭＤＡ比は徐々に増加し、シナプスの成熟化が進んだ。この時期にＡＭＰＡ受容体を介する内側毛帯ＥＰＳＣｓの減衰時間は短縮した。薬理学的な検討からそのシナプス電流の性質変化はＡＭＰＡ受容体のｆｌｉｐ型からｆｌｏｐ型への切り替わりによると推定された。生後第四週以降（Ｐ２１～）においては、内側毛帯繊維の配線、内側毛帯ＥＰＳＣｓの性質に変化は見られなかったことから、内側毛帯シナプスは既に成熟したと考えられた。

次に竹内雄一は、内側毛帯シナプスが成熟した生後第２１日においてマウスの髭の感覚を司る末梢感覚神経（三叉神経第二枝）を切断し、一週間後（Ｐ２８－３２）に対応する領域の内側毛帯ＥＰＳＣｓを記録した。その結果、生後発達初期に見られたような中継細胞に対する多重支配が観察され、内側毛帯繊維の再投射が示唆された。この様な内側毛帯繊維の再投射は髭を抜く操作のみでは引き起こされなかったことから、感覚入力の欠如ではなく末梢神経の損傷が原因であると示唆された。次に切断群と偽切断群において単一の内側毛帯繊維を介するＥＰＳＣｓの性質を比較検討したところ、切断群において、小さいＥＰＳＣｓの振幅および長いＥＰＳＣｓの減衰時間で特徴づけられる内側毛帯繊維群が認められた。この様な性質を持つ繊維群は偽切断群においては認められなかったことから、竹内雄一はこの繊維群が再投射した繊維群であると考え、“Ｗｅａｋ　ｆｉｂｅｒ”と名付けて解析を行った。Ｗｅａｋ　ｆｉｂｅｒを介するＥＰＳＣｓはｓｈａｍ群のＥＰＳＣｓに比して小さいＡＭＰＡ/ＮＭＤＡ比を持っていた。さらに竹内雄一はＡＭＰＡ受容体を介するＥＰＳＣｓについて、ストロンチウムを用いた素量解析、電流電圧曲線解析、および薬理学的解析を行い、Ｗｅａｋ　ｆｉｂｅｒが形成する内側毛帯シナプス後部に視床における幼若型のＧｌｕＲ２サブユニットを含みｆｌｉｐ型のＡＭＰＡ受容体が出現していることを明らかにした。

さらに竹内雄一はこのように神経損傷による視床求心性シナプスの再編成を神経筋接合部、小脳、および大脳皮質のシナプスの報告と比較考察した。その結果一般的に神経系は傷害受けた後に幼若期の表現型に戻り、新たな環境に適応するように回路の再編成を行

うのではないかと結論づけた。

研究目的および手法も適切であり、論理的な内容で学位論文として認められるものである。