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学位論文題目	EXOCYTOSIS AND FILOPODIAL ACTIVITY IN NERVE TERMINALS
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論 文 内 容 の 要 旨

The motile endings of the neurites are the nerve terminals. It is a highly polymorphic and polyfunctional structure and plays a significant role in the functioning of the nervous system. A knowledge of this structure is of fundamental importance for the understanding of the activity of the nervous system. The nerve terminals release and respond to neurotransmitters, elongate, orient the advancing neurite in the right direction, make synaptic contacts with specific target cells and modify these synapes when necessary. All these activities are associated with specific changes in the morphology of the nerve terminal. This review concentrates on the morphological changes associated with two such activities, i e., exocytosis and rapid filopodial formation.

Filopodia of the nerve terminals are believed to play various roles including neurite extension, recognition of target cells, adhesion to suitable substrates, and probably synaptic plasticity. Video enhanced contrast differential interference contrast (VEC-DIC) microscopy and fluorescence microscopy revealed various activity of the growth cone. The extension and slow movement of filopodia, veiling of lamellipodia, advancement of granules to the periphery and constriction of the main body of the growth cone were observed. The treadmilling of actin fibers plays major role in filopodial extension and movement. The structure and properties of the filopodia and the nerve terminal per se are modified by electrical stimulation, treatment with neurotransmitters, presence of calcium in the extracellular medium, and the state of differentiation of the neuron. Filopodia can be induced to form rapidly by electrical stimulation and K-depolarization (within a few hundreds of milliseconds) in the nerve terminal of neuronally differentiated chromaffin cells and PC12 cells. The

growth length reaches 10 μm and the growth rate reaches 2 μm/s. This rapid growth can be suppressed by removal of external Ca ion, addition of La ions or local anesthetics (lidocaine) to the external medium. Repetitive stimulation induced such filopodia much more effectively than single stimulation. These filopodia are transient in nature and very flexible to show Brownian motion (reflecting a sparsity of cytoskeleton). In other cells (hippocampal neurons and astrocytes), glutamate could induce the formation of actin containing filopodia. The mechanism of their formation and their physiological role is unclear at the moment. Since repetitive stimulation is very effective it is our speculation that rapidly induced filopodia may be involved in synaptic plasticity.

Nerve terminals release neurotransmitters by exocytosis. This process involves the fusion of secretory granule membrane with plasma membrane and the formation of the omega figure observed by electron microscopy. Evidence for the fusion was also obtained from biochemical and immunohistochemical studies which showed addition of granule membrane to the cell surface membrane. VEC-DIC microscopy is an effective method to study the dynamics of exocytosis at a very high magnification in real time. Exocytosis was seen by VEC-DIC as an abrupt change in the light intensity of secretory granules. In very thin cells, the omega figure can also be captured. This can be seen in isolated nerve terminals of the rat neurohypophysis and neuronally differentiated chromaffin cells in culture. The rate of release of transmitters or hormones from a single granule can be measured from the rapid light intensity change. It ranges from 8 ms to 15 ms.
A very stable baseline of the light intensity record before the abrupt change indicates that the granules or vesicles do not move a distance larger than one tenth the diameter immediately before the membrane fusion process. The video image also indicates that granules do not

swell before the membrane fusion process. Subtraction of a video image from another image of a 300 ms interval shows a faint pattern due to movement of many granules in the nerve terminal. The intensity histogram of pixels in this image reflects the mobility of a group of granules. Such a quantitative comparison of granule movements indicates that each granule tends to slow down or stop moving during the stimulated state. These findings provide evidence against traditional hypotheses for triggering mechanism of granule fusion with the plasm membrane. Continuous time differentiation of image allows counting of exocytotic events in single cells. By image analyses, the frequency of exocytosis and its spatial distribution in nerve terminals can be studied. Application of K-rich solution induces a persistent response of exocytosis. Repetitive electrical stimulation induces facilitated responses of exocytosis. The frequency of exocytosis is very high especially in the region near the terminal-cell contact. When the frequency of exocytosis is high, a small omega figure made after an exocytotic response of a single granule grows to a large caveola and serves as an enlarged plasm membrane to provide a large fusion site, thereby facilitates the exocytotic responses. These investigations of the morphological changes associated with the neuronal events provide clues to the molecular mechanism underlying these events.

論文の審査結果の要旨

論文は副腎クロム親和細胞とＰＣ１２細胞を培養しニューロンの軸作突起様に分化した部分をノマルスキー型倒立顕微鏡を用いて観察し、超高倍率高分解能でビデオに記録し、開口放出像と糸状仮足突起との関係を動的に分析したものである。神経末端への電気刺激によって糸状仮足(Filopodia) に似た構造が出現する動的な現象の記述は、新しい発見として評価できる。糸状仮足の発現に関する刺激実験、外液カルシウムや薬物の効果についても明快な記述である。観察された現象とシステムは大変興味深い。培養神経細胞の突起終末端の生理活動に関連した事項のレビューは広範囲でていねいである。引用文献数も多くページ数も十分で大学院生として要求される一般知識も十分身につけていることが伺われる。英文は構成に多少の問題も見られるが、概して読み易く正確である。電気刺激によって発現する糸状仮足の発芽機構についての解析は今後の問題であり、分子レベルでの解析、細胞内カルシウム反応との関連、細胞内骨格との関連等の研究が残されているが、現段階で、提出論文そのものは全体として独創的な実験結果を含むレビューとして価値が高いと判定できる。

学生自身による提出論文の解説はビデオを多用し分かりやすいものであった。言語は全て流暢な英語で高い会話能力も示された。全ての質問に妥当な返事をすることができた。今までに記述のなされていない新しい現象であるだけに、「糸状仮足」の語を使うことが適当かどうかが問題とされたが、その動的な特徴が今まで知られていた糸状仮足のものと違うことを除けば、形態的にはほとんど区別がつかず、糸状仮足の新しい形として受け入れることは可能という結論に達した。

以上に基づき、平成４年２月７日に開催された生理科学専攻委員会は、主査らによる審査経過概要の説明をし、さらに審議を重ねた結果、シャンティ・マニバナン提出の論文を大学院博士論文として受理した。