〔Original〕

Brain pathology of Lafora disease : localization of polyglucosan aggregations (Lafora bodies) within neuronal networks of the cerebral cortex

Noriaki Yoshimura [1)]

Abstract

Lafora disease (LD) is an autosomal recessively inheritable metabolic disorder of carbohydrate, characterized clinically by myoclonus, epileptic convulsions and mental deterioration, and pathologically by widespread presence of polyglucosan bodies (Lafora bodies; LB) in the CNS neurons and the cells of other organs. Although the histochemistry and ultrastructure of LB have been well reported, localization of LB within neuronal networks has not been well clarified yet. The purpose of the present study is to elucidate this issue. A male patient with LD who died at 30 years of age was autopsied. The autopsy case was reported elsewhere. This time, specimens taken from the frontal and temporal cortices were observed by electron microscopy. There were a number of synapses on the cell membrane which covered large LB within the perikaryon and dendritic shaft. LB were encountered at times in myelinated axons. Very small LB (ø <2 μm) were occasionally found in the presynaptic terminals of asymmetric synapses, but hardly found in the postsynaptic endings. It is known that disrupting the delicate balance of inhibitory and excitatory synaptic transmission can trigger the disordered, synchronous firing of neurons that underlies a seizure. The results of the present study strongly suggest that disruption of the delicate balance of such synaptic transmissions, which can trigger the disordered synchronous discharges of neurons, may well take place in the cerebral cortices in LD.

Key words: Lafora bodies, localization within neuronal networks, synapses, myoclonus epilepsy, pathogenesis

Introduction

Lafora disease (LD) is an autosomal recessively inheritable metabolic disorder of carbohydrate, characterized clinically by myoclonus, epileptic convulsions and progressive mental deterioration, and pathologically by widespread presence of polyglucosan bodies (Lafora bodies; LB) in the CNS neurons and cells of other organs. The disease manifests with stimulus-sensitive tonic-clonic, grand mal, visual and myoclonic seizures which rapidly progress to severe myoclonic epilepsy often followed by status epilepticus. Patients with LD result in dementia, muscle wasting and respiratory failure. The disease mostly occurs between the age of 10 and 17 and death follows about 10 years after the onset (Anraku and Hakusui, 1989; DiMauro, 1996; Lafora and Glueck, 1911; Namba 1978; Seitelberger, 1968).

The histopathological hallmark of LD is LB which widely appear not only in the brain but also in many other parenchymal tissue cells such as those of the liver, myocardium, skeletal muscle and dermal sweat glands, in addition to smooth muscle of the digestive tract and epithelium and/or myoepithelium of the prostatic gland (Adams and Lee, 1982; Carpenter and Karpati, 1981; DiMauro, 1996; Seitelberger 1968; Thom et al, 2008; Yoshimura et al, 1999). LB in the brain are seen predominantly in perikarya and dendrites of neurons.

1) Brain Pathology and Medical Neuroscience, Hirosaki University of Health and Welfare
3-18-1 Sanpinai, Hirosaki 036-8102, Japan

The majority of the LB which stained strongly with periodic acid-Schiff (PAS) have a darkly stained core and (a) lightly stained and occasionally radiating shell(s). The larger LB are invariably perikaryonal or thick-dendritic in a distribution similar to that of endoplasmic reticulum. On the other hand, sandgrain-like granules which are only revealed clearly by PAS staining are concentrated mostly in neuronal small dendritic processes and on rare occasions in thin axons (Adams and Lee, 1982; Iwata 1986; Seitelberger, 1968; Van Haycop Ten Ham, 1974; Yagishita, 1994; Yoshimura et al, 1999; Yoshimura, 2005).

Cytochemical studies revealed that LB contained a protein moiety which appeared to be sensitive to both chymotripsin and pepsin, but more specifically to the latter (Gambetti et al, 1971; Nikaido et al, 1971). LB contain 80-93% polyglucosan and 6% proteins depending on tissues (Yokoi et al, 1968; Sakai et al, 1970). Although the natures of the proteins have been believed to be mostly unknown, recent studies (Criado et al, 2011; Aguado et al, 2010; Olzmann et al, 2008) may suggest that they have something to do with the dysfunction of autophagy which results in the failure of clearance of aggresomes (Yoshimura 2012). Polyglucosans are malformed glycogen molecules (glycose polymers) that appear as peculiar linear strands defective of normal branching (DePaoli-Roach et al, 2010). Resembling amylopectin, polyglucosans are poorly soluble hence precipitate inside cells. Accumulated precipitations form peculiar filaments, i.e. polyglucosan filaments, whose aggregations build LB.

LD is caused by mutations in either the EPM2A gene present on 6q24, encoding laforin (Minassian et al, 1998; Minassian 2001), or the EPM2B gene located on 6p22.3 which encodes malin (Chan et al, 2003; Chan et al, 2003), although there is evidence for a third locus (Chan et al, 2004). Laforin is a glycogen phosphatase (a dual specificity protein phosphatase with a functional carbohydrate-binding domain). Malin is an E3-ubiquitin ligase having RING finger domain at the N-terminus. However, the disease mechanisms that are brought about by mutations in these two genes are poorly understood so far. Therefore, it is necessary to elucidate what the structural and functional changes are expressed on cell and tissue structures *in vivo*, due to the defective gene product.

The fine structural changes of LB have been reported by many authors so far (Anraku and Hakusui,1989; Collins et al, 1968; Van Haycop Ten Ham, 1974; Vanderhaeghen, 1971; Cajal et al, 1974; Gambetti et al, 1971; McMaster et al,1979; Oyanagi 1992; Van Hoof Hageman-Bal,1967; Yoshimura et al, 1999; Yoshimura 2012). Almost all of them, however, have reported on those of the matured/developed phase of LB rather than their early or developing phase. It has been reported that the lack of laforin-malin complexes causes the dysfunction of autophagy, which plays a primary role in the LB formation (Criado et al, 2011; Aguado et al, 2010; Olzman et al, 2008).

Recently, after classifying LB into 3 phases, i.e. early, developing, and developed phases by definite standards which were obtained from both findings of PAS stain and of EM, the author has observed LB in 3 individual phases, and has found that the fine structural changes of LB in the developing phase could be the key findings that link to the underlying molecular mechanisms of not only LB formation but also LD development (Yoshimura 2012). Although the histochemistry and ultrastructure of LB have been fully reported (Seitelberger, 1968; Nikaido et al., 1971; Yokota et al, 1987; Oyanagi, 1992; Yoshimura et al,1999; 2012), localization of LB within neuronal networks have not been reported yet. To elucidate the localization of LB is considered to be important not only from the view point of generation of clinical symptoms such as epileptic seizures followed by dementia, but also from that of LD pathogenesis. The purpose of the present study is to elucidate this issue.

Objects and Methods

The male patient with LD with a 17-year clinical course, who died at 30 years of age, was autopsied 8 hours after death. The brain and the serial sections showed no gross abnormality except for a slight increase in weight (1500g) and clear depigmentation of the substantia nigra of both sides (Figs.1a & 1b).

The general findings including those of the CNS and visceral organs were reported elsewhere (Yoshimura et al, 1999). EM tissue specimens were taken at autopsy from frontal, temporal, and occipital cortices in addition to substantia nigra, putamen, inferior olivary nucleus, cerebellar cortices and so on. These epon-embedded tissue blocks had been made

immediately after the autopsy. This time, after re-observing the preparations used for routine neuropathological examinations of this case (Figs.1a-e), semi-thin sections stained with 1% toluidin blue solution were made from the cerebral cortices of the above-described specimens. After observing the toluidin blue stained semi-thin sections, ultrathin sections which were obtained by using a Porter-Blum ultramicrotome (MRC; MT-6000) were double-stained with uranyl acetate and lead citrate. They were examined by an H-600 electron microscope (Hitachi, Tokyo, Japan) at 80kV.

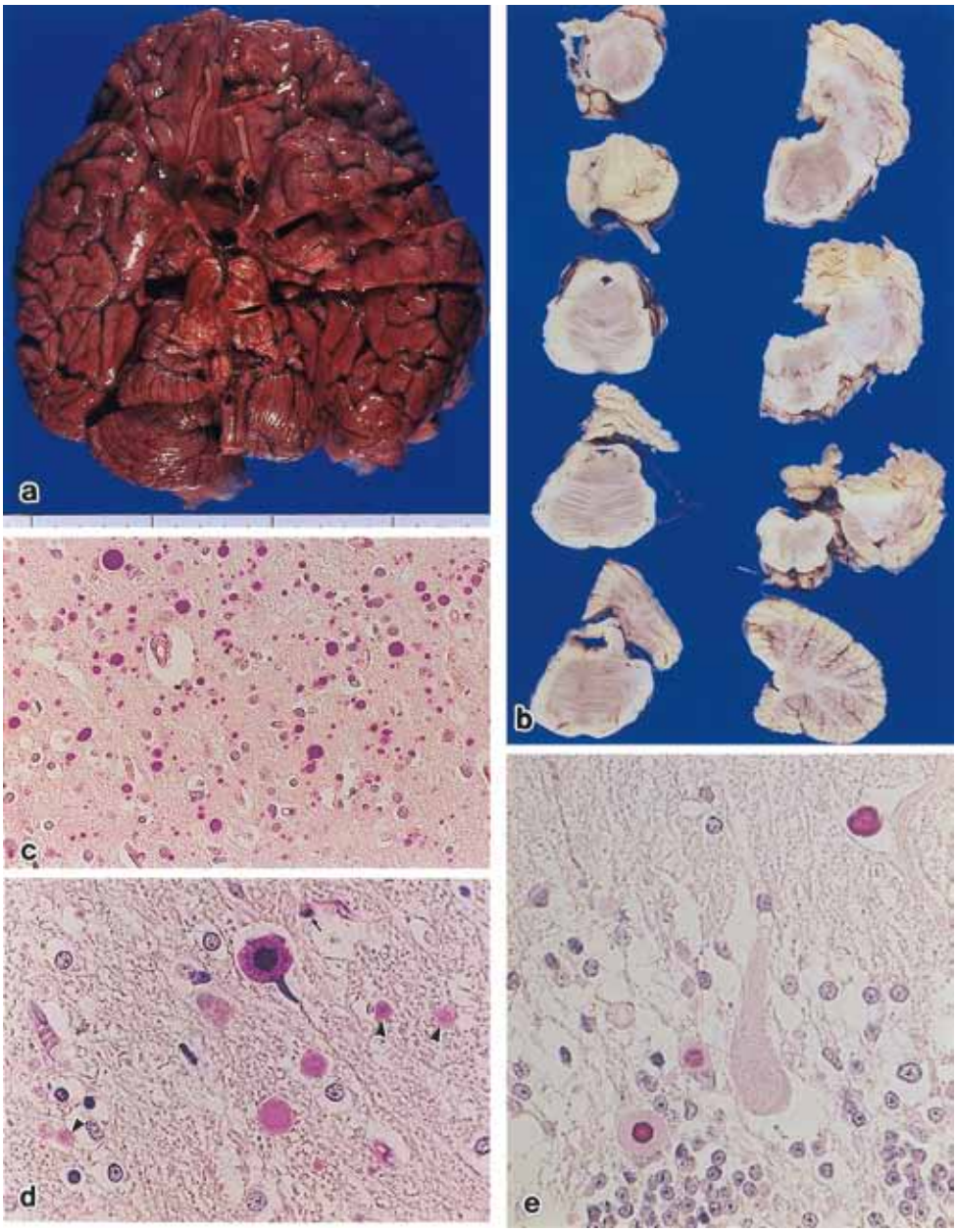

Fig.1
a. Basal view of the fresh brain before fixation showing no gross abnormality except for slight atrophy of the brain stem and cerebellum.
b. Serial sections of the brain stem and cerebellum showing obvious depigmentation of the substantia nigra.
c. Various profiles of LB: (a) concentric LB, (b) cored LB with a radiating light rim, (c) light or dark homogeneous LB, and (d) sand-grain like LB whose diameters were around 5 μm. Calcarine cortex, PAS x 260.
d. Besides a cored LB with the radiating shell in a neuron and 2 dimly concentric LB within neurons, 3 intra-astrocytic (*arrowheads*) and 1 intra-microglial or pyknotic astrocytic (*arrow*) LB are detectable. Red nucleus. PAS x 520.
e. Other than 3 typical LB in 3 neurons, two homogeneous and small LB in glial cells can be seen. Cerebellar cortex PAS x 520.

Results

Before EM observations, careful re-examinations of PAS-stained slides by light microscopy revealed that aggregations of polyglucosans were occasionally present within glia, mostly astrocytes (Fig.1d). The cells which bore polyglucosans within the cell bodies were recognized to be astrocytes by their much smaller and less clear cellular and nuclear contours, which contained a small amount of pinkish polyglucosans that had the dim outline, in contrast to those of nerve cells (Fig.1d).

Light homogeneous LB (with PAS stain) showed that the entire area of the light homogeneous body was composed exclusively of compactly intermingled filaments, associated with a small number of dense granules. The more the dense granules deposited diffusely and densely, the darker the homogeneous LB became, and resulted in dark homogeneous LB (Yoshimura, 2012). When the dense granule deposition localized centrally or ringedly, the light homogeneous LB would become light homogeneous LB with a central core or with ring(s).

There were a number of chemical synapses, mostly

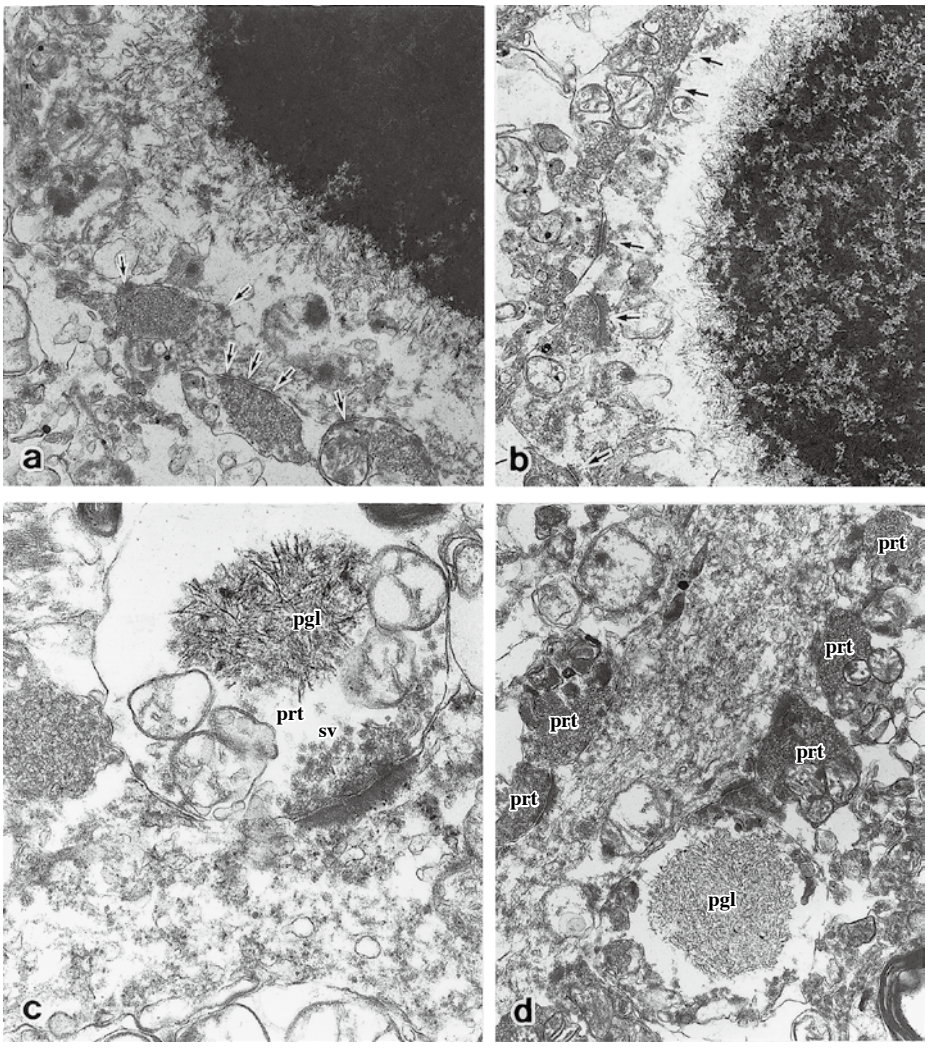

Fig.2 **a** and **b.** There are a number of synapses (*arrows*) on the cell membrane which covered large LB within the perikaryon of a neuron. Superior temporal cortex. **a.** x 12,000 **b.** x 10,000.

c. Aggregation of polyglucosan (*pgl*) material to form branchless straightened filaments 6-8nm in diameter in a presynaptic terminal (*prt*) of an asymmetric synapse. Superior frontal cortex. x 20,000.

d. In some of axo-somatic and/or axo-dendritic synapses, the presynaptic terminals (*prt*) were extraordinarily increased in density and packed with synaptic vesicles (*sv*), a few mitochondria and some degeneration products, in addition to those containing a compact mass of polyglucosan (*pgl*)filaments. Superior frontal cortex. x 10,000.

symmetric ones, on the cell membrane which covered large LB within the perikaryon and/or thick part of dendrites (Fig.2a-d). In these axo-somatic and/or axo-dendritic synapses, the presynaptic terminals were sometimes extraordinarily increased in electron-density and packed with synaptic vesicles, a few mitochondria and some degeneration products (Fig.2a, 2b and 2d). The terminals occasionally contained an aggregation of polyglucosan fibrils (LB), which was mostly composed of fine irregular and poorly branched filaments with about 6-8nm in diameter, often associated with a variable number of amorphous dense granules (Fig.2c, 2d and 3a-d). The LB in the synaptic terminals (micro-LB) were very small and mostly measured less than 2μm in diameter. Micro-LB were occasionally found in presynaptic terminals and scarcely found in the postsynaptic endings of dendrites. Micro-LB in presynaptic terminals tended to occur rather in asymmetric synapses (Figs.2c and 3a-c) than in symmetric ones (Fig.3d). Most micro-LB which were found in the present study were formed in asymmetric synapses. On rare occasions, however, sparse accumulation of polyglucosan filaments (LB) in the presynaptic terminal of a symmetric synapse was found.

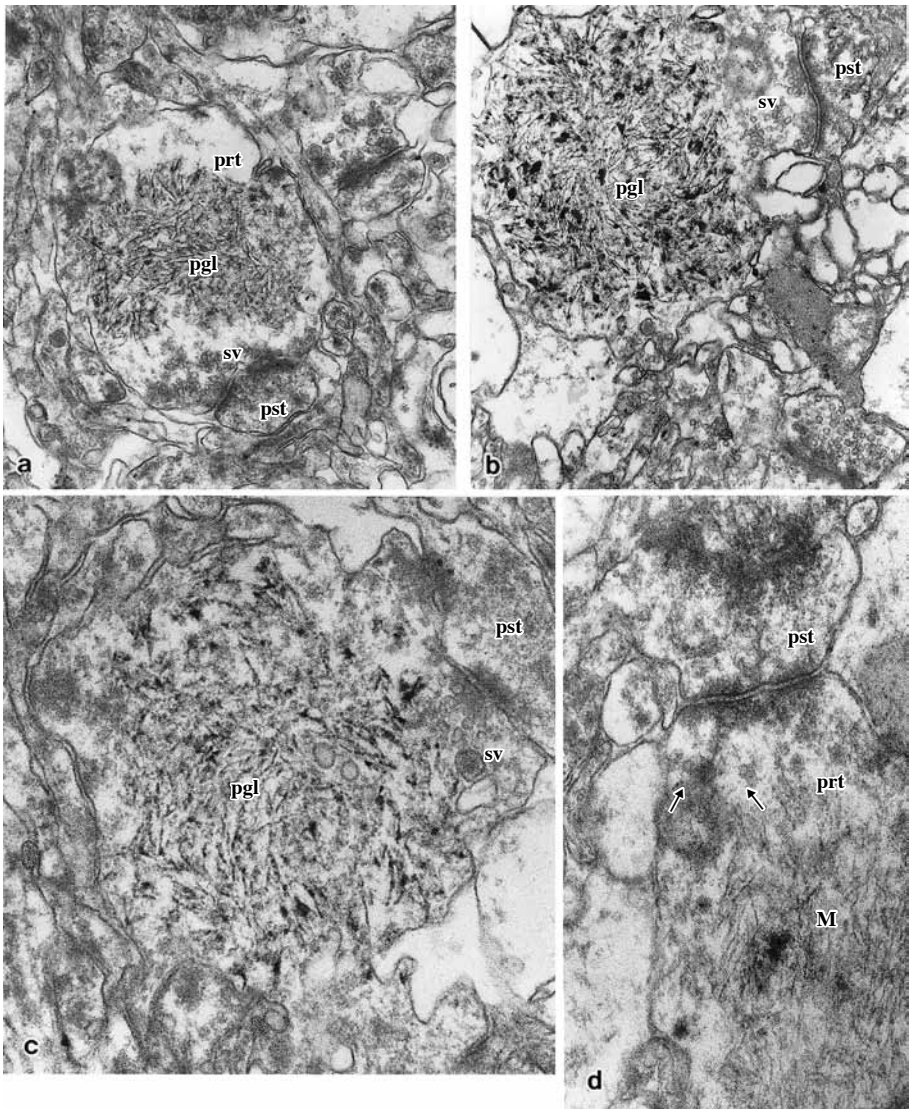

Fig.3 **a-c.** Various profiles of a mass of aggregated polyglucosan filaments (*pgl*) within the presynaptic terminal (*prt*) of an asymmetric synapse. pst; postsynaptic
a. and **b.** Superior temporal cortex x 20,000.
prt; presynaptic pst; postsynaptic sv; synaptic vesicles **c.** Superior frontal cortexx x 35,000.
d. Aggregation forming a loose mass (M) of branchless straightened filaments, which may be a precursor of LB in a presynaptic terminal (*prt*) . Arrows indicate synaptic vesicles (*sv*).
Superior frontal cortex. x 20,000.

In one occurrence, the filaments were rather thin, fine and rigid-looking, suggesting a very early phase of an aggregation of polyglucosan filaments (an incipient LB) (Fig.3d).

A substantial number of small LB whose diameters measured approximately 5μm, corresponding to "sandgrain-like LB" under the light microscope (Fig. 1c), were found (Figs.4a and 4b). It was, however, often difficult to find definite structures of chemical synapses on the cell membrane that covered those small LB (Figs.4a-c). LB were occasionally encountered in myelinated axons (Fig.4d). Their cut-surfaces were always nearly round in shape.

In addition to this study, throughout all observations by both light and electron microscopy which included our previous investigations (Yoshimura et al, 1999; Yoshimura 2012), it has been revealed that every cut-surface of LB except for those of very early phase (Fig.3d) has been nearly round in shape. Such changes as curly fibers in Alzheimer's brains or Lewy neurites in Lewy body diseases that indicate the growth of longitudinal direction of the storage materials (i.e. tau or α-synuclein) within the neurites has not been observed.

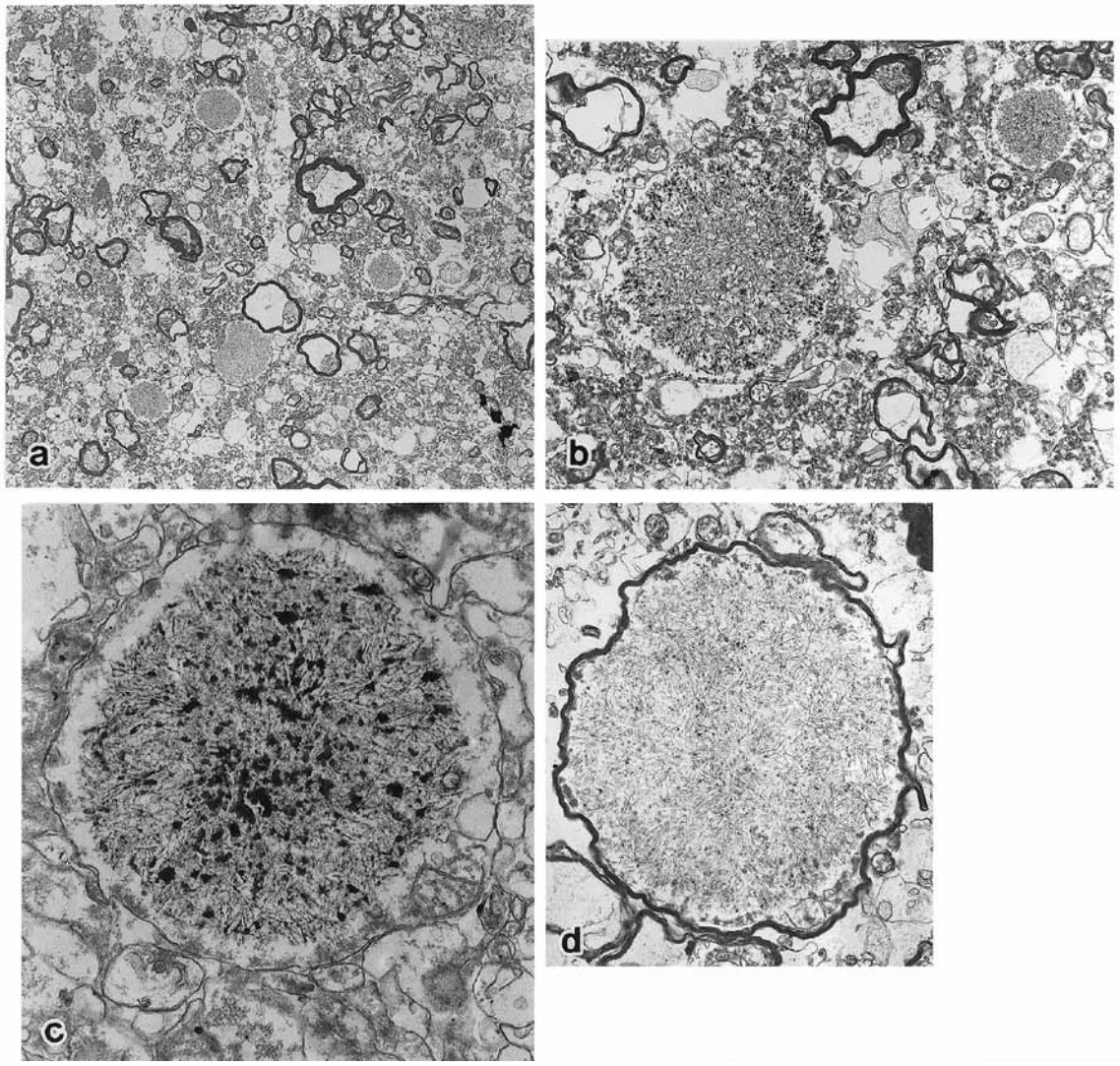

Fig.4 **4a.** Three sand-grain like LB whose diameters are all shorter than 5μm. There were no definite chemical synaptic structures on the membrane covering these LB. Superior frontal cortex x 2,000.

b and **c.** Higher magnification of a similar sand-grain like LB often showing no definite chemical synaptic structures on the covering membrane. Superior frontal cortex **b.** x 5,000. **c.** x 10,000.

d. An aggregation of polyglucosans (LB) in a myelinated axon, where the axoplasm is almost completely replaced by dense polyglucosan filaments interwoven in various directions. Superior temporal cortex x 6,500.

Discussion

Our results indicate that an aggregation of polyglucosan filaments (LB) can occur in the neuronal perikaryon and thick portions of dendrites, where LB grow bigger and bigger and show various typical profiles (such as, light homogeneous LB, dark homogeneous LB, cored LB, pin-point cored LB, ringed LB, and so on) that are well-known (Yoshimura, 2012). Where there are wide spaces LB may grow bigger than in narrow spaces. In myelinated axons and thin portions of dendrites, LB grow usually homogeneous, with a shorter diameter than that of those grown in the perikaryon and thick portions of dendrites.

In addition, a very small aggregation of polyglucosan filaments (i.e. micro-LB) could sometimes be found in the presynaptic terminals. In the postsynaptic endings of dendrites and/or perikarya, however, micro-LB were hardly encountered (only one could be found), as far as the present study was concerned. The localization of LB, which should correspond to that of LB demonstrated in the present study, was summarized in Fig 5. Gambetti et al. (1971) were the first to illustrate that a small aggregation of polyglucosan filaments can occur both in presynaptic terminals and in postsynaptic dendrites, although they made no detailed comment on the matter. Interestingly in the present study, most of the micro-LB in presynaptic terminals were observed in asymmetric synapses, which are known to release excitatory amino acids, glutamate or aspartate (Kandel et al, 2013; Uchizono, 1977). In one instance, the filaments were rather thin, fine and rigid-looking, suggesting a very early phase of aggregation of polyglucosans to form filaments, which seemed to be about to shape a mass as a LB (Fig.3d). These filaments were very thin and measured approximately 6 nm in diameter. As time lapses in the process of LB formation, i.e. from the very early phase to the developing and developed phases, those filaments are known to be subjected to recurrent phosphorylation (Tagliabracci et al, 2007), which should be responsible for their minimally wider diameters (approximately 8nm) and an increase of rigid-appearance as those seen in LB in the developed phase (Yoshimura 2012).

An emerging concept that was learned from examining Alzheimer's Aβ-peptide has been proposed (Haass & Selkoe, 2007). Namely, a series of brain proteins in misfolded diseases whose soluble oligomers can gain an adverse bioactivity in the aggregation process and affect synaptic structures and plasticity, leading to neural cell death (neurodegeneration). Although the mechanisms by which soluble protein oligomers kill neural cells are not fully understood, a common feature is said to be the concentration of unstructured monomers on bidimensional membrane lattices. Membrane-bound monomers undergo a series of lipid dependent conformational changes, leading to the formation of oligomers of varying toxicity rich in β-sheet structures or in α-helix structures (Fantini & Yahi, 2010). As to polyglucosans, it is tempting to consider that similar events could take place, because polyglucosans are malformed glucose polymers (glycogen molecules) that appear as peculiar linear strands defective of normal branching (DePaoli-Roach et al, 2010). Resembling amylopectin, polyglucosans are poorly soluble hence precipitate inside cells. Hyperphosphorylated filaments of polyglucosans are thought often difficult to be digested completely by amylase, although it is still unknown whether or not polyglucosan oligomers are cytotoxic enough to kill neurons. Cytochemical studies revealed that LB contained a protein moiety which appeared to be sensitive to both chymotripsin and pepsin, but more specifically to the latter (Gambetti et al, 1971; Nikaido et al, 1971). Immunohistochemically, it has been demonstrated that LB show in part a positive reaction against both anti-tau and anti-ubiquitin antibodies (Yoshimura, et al, 1999), and also against HSP72 (Yokota et al, 1987). Biochemical studies disclosed that LB contain 80-93% polyglucosan and 6% proteins depending on tissues (Yokoi et al, 1968; Sakai et al, 1970). Although the natures of the proteins have been believed to be mostly unknown, recent studies (Criado et al, 2011; Aguado et al, 2010; Knecht et al, 2010; Rao et al, 2010; Olzmann et al, 2008; Mittal et al, 2007) may suggest that they have something to do with the dysfunction of autophagy which results in the failure of clearance of aggresomes.

The present study demonstrated the multiple presence of intra-axonal LB within the cerebral cortex of the patient. This indicates that for the patient epileptic convulsions was ready to be inducible spontaneously, because there are some studies which demonstrated that epileptic convulsions can be induced when axonal flows of cerebral cortical neurons are

disrupted (Gibbs and McNamara 2006). In addition, the present study revealed the multiple occurrence of a very small mass of polyglucosan filaments (i.e. micro-LB) in the presynaptic terminals, especially in the asymmetric synapses and also in postsynaptic endings of cerebral cortical neurons. This indicates that the disruption of the delicate balance of inhibitory and excitatory synaptic transmission can be induced, which is known to trigger the disordered, synchronous firing of neurons that underlies a seizure (Gibbs & McNamara, 2006). The results of the present study strongly suggest that disruption of the delicate balance of such synaptic transmissions, which can trigger the disordered, synchronous discharge of neurons, may well take place in the cerebral cortex in LD.

A substantial number of very small LB whose diameters measured approximately 5μm, corresponding to "sandgrain-like LB" under the light microscope (Fig. 1c) were found electron microscopically (Figs.4a and 4b). Curiously however, it was sometimes difficult to find definite synaptic structures on the cell membrane that covered such small LB (Figs.4a-c). Since the part of neurites which contained such LB showed distension more or less, the actual diameter of the part would be approximately shorter than 5μm. There seems to be no record that there can be any parts of neurites that have no synaptic apparatus, except for the portion of axon-dividing terminals. As described above, re-

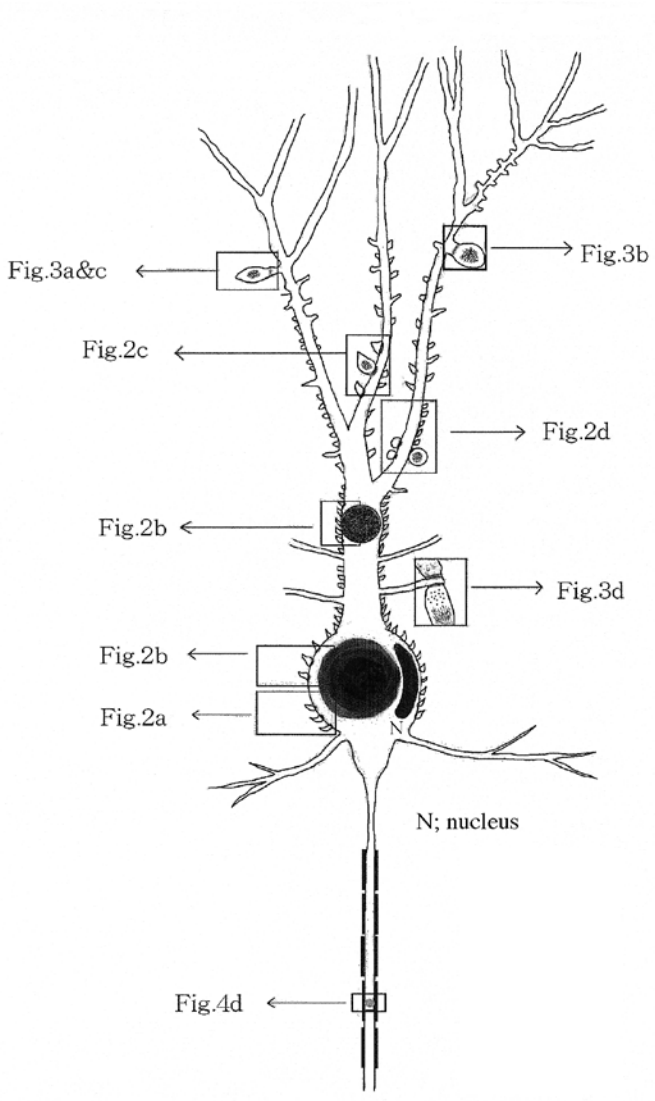

Fig.5 A summerized illustration of LB localization within a neuron which should correspond to that of each LB demonstrated in the present study.

examinations of PAS-stained slides which were made before EM observations revealed that aggregations of polyglucosans were at times present within glia, mostly astrocytes (Fig.1d). The cells bearing polyglucosans within the cell bodies were recognized as astrocytes by their much small and unclear cell bodies and nuclei with containing pale pinkish small amount of polyglucosans that had the dim outline, in contrast to those of nerve cells (Fig.1d). However, this should be reconfirmed by more reliable methods such as double staining with PAS and anti-GFAP antibody.

There were a substantial number of axo-dendritic presynaptic terminals which extraordinarily increased in density and were packed with synaptic vesicles, a few mitochondria and some degeneration products (Figs.2d). These changes of the presynaptic terminals indicate that there was strong damage on the cell bodies and/or proximal axons of the neurons (Tsukahara 1983). As one of such causative factors, ischemia and/or anoxemia due to frequent attacks of epileptic convulsions may be taken into account. Under these conditons, precipitation, aggregation and filament generation of polyglucosans followed by the formation of micro-LB to large LB within the perikarya, dendrites, axons and pre-and post-synaptic endings could be facilitated as a vicious circle, and pathologic signal inputs to those structures, which may also be one of the triggering factors for epileptic convulsions. In the case of Taylor-type focal cortical dysplasia, dilatation of postsynaptic dendritic spines and shafts was one of the fine structural features that were considered to have something to do with the cause (dysgenetic changes) and result (subsequent degenerative processes) of this intractable epilepsy (Kakita et al, 2005). In the Lafora specimens exanined, however, postsynaptic dendritic spines were not remarkably dilated but rather atrophic or hypoplastic (Fig.3a-d). Instead, as described above, presynaptic terminals were sometimes dilated and occasionally contained polyglucosan aggregations (Figs.2c, d and Fig.3a).

Finally, our previous study (Yoshimura et al, 1999) revealed the almost maximum range and distribution pattern of LB in the brain and general organs of a patient with a 17-year clinical course, who died at 30 years of age. The maximum range and distribution pattern of LB in the brain may represent the relationship between the degree and brain locations that have much to do with glycogen metabolism. The present study has demonstrated a possibility that there will be multiple and simultaneous occurrence of LB not only in synapses and axons but also in the perikaryon, dendritic shafts and branches of individual cortical neuron that has more to do with glycogen metabolism (Fig.5).

Acknowledgements

The main data of this study were obtained in 2009. The author expresses his heartfelt gratitude for those who gave him support and offered their kindness as well. The electron microscope was used in Hirosaki University Graduate School of Medicine.

(*Accepted*: February 27, 2013)

References

1. Adams RD, Lee JC: Neurons and neuronal reaction in disease states. In: Haymaker W and Adams RD (edt) Histology and Histopathology of the Nervous System. Charles C Thomas, Springfield, USA, pp.174–275, 1982.
2. Aguado C, Sarkar S, Korolchuk VI, Vernia S, Boya P, Sanz Pscual, de Cordoba SR, Knecht E, Rubinsztein DC: Laforin, the most common protein mutated in Lafora disease, regulates autophagy. Hum Mol Genet 19(4): 2867–2876, 2010.
3. Anraku S, Hakusui M: Lafora disease. In: Naito H, Oyanagi S (eds) Progressive myoclonus epilepsy. Tokyo, Igakushoin, 25–48, 1989.
4. Cajal SRy, Blanes A, Martinez A, Saenz E, Gutierrez M: Lafora disease: an ultrastructural and histochemical study. Acta Neuropathol. (Berl.) 30: 189–196, 1974.
5. Carpenter S, Karpati G: Sweat gland duct cells in Lafora disease:diagnosis in skin biopsy. Neurology 31: 1564–1568, 1981.
6. Chan EM, Bulman DE, Paterson AD, Turnbull J, Andermann E, Andermann F, Rouleau GA, Delgado-Escueta, Scherer SW, Minassian BA: Genetic mapping of a new Lafora progressive myoclonus epilepsy locus (*EPM2B*) on 6p22. J Med Genet 40: 671–675, 2003.
7. Chan EM, Omer S, Ahmed M, Bridges LR, Bennet C, Scherer S.W, Minassian BA: Progressive

myoclonus epilepsy with polyglucosans (Lafora disease): evidence for a third locus. Neurology 63: 565–567, 2004.
8. Chan EM, Young EJ, Ianzano L, Munteanu I, Zhao X, Christopoulos CC, Avanzini G, Elia M, Ackerley CA, Jovic NJ, Bohlega S, Andermann E, Rouleau GA, Delgado-Escueta AV, Minassian BA, Scherer SW : Mutations in *NHLRC1* cause progressive myoclonus epilepsy. Nat Genet 35: 125–127, 2003.
9. Collins GH, Cowden RR, Nevis AH: Myoclonus epilepsy with Lafora bodies: an ultrastructural and cytochemical study. Arch Pathol 86: 239–254, 1968.
10. Criado O, Aguado C, Gayarre J, Duran-Trio L, Garcia-Cbrero AM, Vernia S, Millian BS, Heredia M, Roma-Mateo C, Mouron S, Juana-Lopez L, Dominiquez M, Navarro C, Serratosa JM, Sanz P, Bovolenta P, Knecht Ervin, deCordova SR, : Lafora bodies and neurological defects in malin-deficient mice correlate with impaired autophagy. Hum Mol Genet December 20, 2011.
11. DePaoli-Roach AA, Tagliabracci VS, Segvich DN, Meyer CM, Irimia M, Roach PJ: Genetic depletion of malin E3 ubiquitin ligase in mice leads to Lafora bodies and the accumulation of insoluble laforin. J Biol Chem 285(33): 25372–25381, 2010.
12. DiMauro S: Disorders of carbohydrate metabolism. In: Rowland LP (ed). Merrit's Textbook of Neurology, 9th edn. Baltimore: Williams & Wilkins, 1996. 572–575.
13. Fantini J, Yahi N: Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases. Expert Rev Mol Med. 2010 Sep 1; 12: e27.
14. Gambetti P, Dimauro S, Blume RP: Myoclonus epilepsy with Lafora bodies. Arch Neurol 25: 483–493, 1971.
15. Gibbs JW, McNamara JO: The Epilepsies: Phenotypes and Mechanisms. In: Siegel GJ, Albers RW, Price DL (eds) Basic Neurochemistry. 7th Ed Elsevier Academic Press, London, pp.629–638, 2006.
16. Haass C, Selkoe DJ. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol 8: 101–112.
17. Iwata M : Lafora body. Neurol Med (Tokyo) 24: 443–449, 1986.
18. Kakita A, Kameyama S, Hayashi S, Masuda H, Takahashi H: Pathologic features of dysplasia and accompanying alterations observed in surgical specimens from patients with intractable epilepsy. J Child Neurol. 20 (4): 341-350, 2005.
19. Kandel ER, Schwarz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ (Edt): 10. Synaptic integration in the central nervous system. In Principles of Neural Science, 5th Ed, The McGaraw-Hill Co. Inc., pp.210–235, 2013.
20. Knecht E, Augado C, Sarkar S, Korolchuk VI, Criado-Garcia O, Santiago V, BoyaP, Sanz P, deCordoba SR, Rubinsztein DC: Impaired autophagy in Lafora disease. Autophagy 6(7): 991–993, 2010.
21. Lafora GR, Glueck B : Beitrag zur Histopathologie der myoklonischen Epilepsie. Z. Gesamte Neurol Psychiatr 6: 1–14, 1911.
22. Lewis PD, Evans DJ, Shambayati B: Immunohistochemical and lectin–binding studies on Lafora bodies. Clin Neuropathol 9(1): 7–9, 1990
23. MacMaster KR, Powers JM, Henningar GR, Wohltmann HJ, Farr GH. Nervous system involvement in type IV glicogenosis. Arch Pathol Lab Med 103: 105–111, 1979.
24. Minassian BA: Lafora's disease: towards a clinical, pathologic, and molecular synthesis. Pediatr Neurol 25: 21–29, 2001.
25. Minassian BA, Lee JR, Herbrick JA, Huizenga J, Soder S, Mungall, Dunham I, Gardner R, Fong CY, Carpenter S, Jardim L, Satishchandra P, Anderman E, Snead OC, Lopes-Cendes I, Tsui LC, Delgado-Escueta AV, Rouleau GA, Scherer SW: Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. Nat. Genet 20: 171–174, 1998
26. Mittal S, Dubey D, Yamakawa K, Ganesh S: Lafora disease proteins malin and laforin are recruited to aggresomes in response to proteasomal impairment. Hum Molec Genet 16(7): 753–762, 2007.
27. Namba M: Myoclonus epilepsy (Lafora type). Neurol Med (Tokyo) 9: 1–10, 1978.
28. Nikaido T, Austin J, Stukenbrok H: Studies in myoclonus epilepsy. III. The effects of amylolytic enzymes on the ultrastructure of Lafora bodies. J Histochem Cytochem 19(6): 382–385, 1971

29. Olzmann JA, Li L, Chin LS: Aggresome formation and neurodegenerative diseases: therapeutic implications. Curr Med Chem 15(1): 14 pages, 2008.
30. Oyanagi S: A guide to neuropathology by electron microscopy. Igaku-shoin, Tokyo, 1992.
31. Rao SNR, Maity R, Sharma J, Dey P, Shanker SK, Satishchandra P, Jana NR: Sequestration of chaperones and proteasome into Lafora bodies and proteasomal dysfunction induced by Lafora disease-associated mutations of malin. Hum Mol Genet 19(23): 4726–4724, 2010.
32. Sakai M, Austin J, Witmer F, Trueb L: Studies in myoclonus epilepsy (Lafora body form). II. Polyglucosans in the systemic deposits of myoclonus epilepsy and in corpora amylacea. Neurology (Mineap) 20: 160–176, 1970.
33. Seitelberger F: Myoclonus body disease. In: Minkler J (ed) Pathology of the Nervous System, Vol.1. New York, McGraw-Hill, pp.1121 –1134, 1968.
34. Seratosa JM, Delgado-Escueta AV, Posada I, Shih S, Drury I, Berciano J, Zabala JA, Antunez NC, Sparkes RS: The gene for progressive myoclonus epilepsy of the Lafora type maps to clomosome 6q. Hum Mol Genet 4: 1657–1663, 1995.
35. Tagliabracci VS, Turnbull J, Wang W, Girard J-M, Zhao X, Skurat AV, Skurat AV, Delgado-Escueta AV, Minassian BA, DePaoli-Roach AA, Roach PJ: Laforin is a gloycogen phosphatase, deficiency of which leads to elevated phosphorylation of glycogen in vivo. PNAS 104(49): 19262–19266, 2007.
36. Thom M, Sisodiya S, Najm I : Neuropathology of epilepsy. In : Love S, Louis DN, Ellison DW (eds) Greenfield's Neuropathology. Vol.I. Edward Arnold, London, pp.833–887, 2008.
37. Tsukahara N: Memory and synapses. Science [Japanese] 53(12): 762–770, 1983.
38. Uchizono K: Chemical transmission in the nervous system. In (Kuriyama K ed) Structure and Function of Synapses. Igaku-shoin Inc., Tokyo, pp1–23, 1977.
39. Vanderhaeghen J-J: Correlation between ultrastructure and histochemistry of Lafora bodies. Acta Neuripathologica 17(1): 24–36, 1971.
40. Van Hoof F, Hageman-Bal M: Progressive familial myoclonic epilepsy with Lafora bodies. Electron microscopic and histochemical study of a cerebral biopsy. Acta Neuropathol (Berl) 7: 315–326, 1967.
41. Vanderhaeghen, J.-J.: Correlation between ultrastructure and histochemistry of Lafora bodies. Acta neuropath. (Berl.) 17, 24–26, 1971.
42. Van Heycop Ten Ham MW : Lafora disease: a form of progressive myoclonus epilepsy. In : Vinken PJ, Bruyn GW(eds) Handbook of Clinical Neurology, Vol.15. Amsterdam:North Holland, pp.382–422, 1974.
43. Yagishita S: Polyglucosan bodies and dementia. Dementia Japan 8: 78–86, 1994.
44. Yokoi S, Austin J, Witmer F, Sakai M: Studies in myoclonus epilepsy (Lafora body form). I. Isolation and preliminary characterization of Lafora bodies in two cases. Arch Neurol (Chicago) 19: 15–33, 1968.
45. Yokota T, Ishihara T, Kawano H, Yamashita Y, Takahashi M, UchinoF, Kamei T, Kusunose T, Yamada M and Matsumoto N : Immunological homogeneity of Lafora body, corpora amylacea, basophilic degeneration in heart, and intracytoplasmic inclusions of liver and heart in type IV glycogenosis. Acta Pathol Jpn 37: 941–946, 1987.
46. Yoshimura N: Brain Pathology of Lafora disease: fine structural changes of Lafora (polyglucosan) bodiesin the developing phase of the formation. J Hirosaki Univ Health Welfare 3(1): 19–32, 2012.
47. Yoshimura N: Lafora disease. Clin Neurosci 23(2): 132–133, 2005.
48. Yoshimura N, Kaneko S, Yoshimura I, Yamagata K, Ichinohe H, Hagiwara C, Kusumi T, Kudo H: Distribution and electron microscopical and immunohistochemical aspects of Lafora bodies in a Lafora patient with 17-year clinical course. Neuropathol 19 :273–282, 1999.

ラフォラ病の脳病理 ：大脳皮質神経回路網内における ポリグルコサン凝集体（Lafora小体）の局在

吉 村 教 暉[1)]

1）弘前医療福祉大学 脳病理学・病態神経学
〒036-8102 弘前市小比内 3-18-1

要　　旨

La病は常染色体劣性遺伝の糖質代謝障害でてんかんを繰り返し認知症が進行する。その脳細胞にはLB（La小体）が広範に出現するが、LBのニューロン網内局在の十分な報告はまだない。本研究はこの点を調べた。今回は30歳の既報La病剖検例の前頭葉と側頭葉の主にシナプスを詳細に電顕観察し新たな発見をした。①神経細胞核周部や樹状突起内の大型LBを覆った細胞膜上には主に対称性シナプスが幾つも認められた。②LBは時々有髄軸索内に認められた。③微小LB（ø＜2μm）は非対称性シナプス前終末に稀ならず、シナプス後終末には極稀に見られた。④星状膠細胞様細胞核周部にも時に少量のpolyglucosan（PG）凝集体が認められた。これより、神経細胞の核周部、樹状突起近位部・遠位部、および有髄軸索内にPGが析出し、LBとなり、更に興奮性のシナプス終末では微小塊または細線維群として少なからず出現することが証明された。抑制性と興奮性のシナプス伝達の微妙なバランスが撹乱されるとき、発作の基礎となるニューロンの病的同期性発火が誘発されることが知られている。本研究で示されたシナプス終末部におけるPGBの微細線維状構造の出現はこのようなシナプス伝達の微妙なバランスの撹乱を惹起する可能性を十分に示唆する変化といえる。

キーワード：ラフォラ小体（LB / PG B）、神経回路網、シナプス、ミオクローヌスてんかん、発症機序