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# Age changes of neuromuscular junctions in the extensor digitorum longus muscle of spontaneous thymoma BUF/Mna rats

## A scanning and transmission electron microscopic study

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**Abstract** BUF/Mna rats spontaneously develop thymomas and cause muscle weakness of hind legs at an advanced age. This rat strain has been recognized as a suitable animal model for human myasthenia gravis or related myopathies. To characterize the structural changes of neuromuscular junctions (NMJs) in BUF/Mna rats, subneural apparatuses (SNAs) of extensor digitorum longus muscles of young-adult (4-month-old) and aged (22- to 25-month-old) male rats were examined using scanning and transmission electron microscopy. The SNAs of NMJs in young rats consist of complex labyrinthine gutters with numerous slit-like junctional folds. SNAs in aged BUF/Mna rats, however, are characterized by: (1) a group of cup-like depressions with very wide slit-like junctional folds in relatively large muscle fibers (the major type), (2) the presence of slit-like folds on the flat sarcoplasm outside the cup-like depressions or on the protruded sarcoplasm, and (3) winding gutters or a small number of round depressions with poorly developed synaptic folds in small and medium-sized muscle fibers (the minor type). Since similar structural changes have been reported in dystrophic mice or normally aged rats, it is suggested that both the slowly progressing muscle atrophy and age-dependent turnover of muscle fibers may occur in the aged BUF/Mna rats.

**Keywords** Muscle weakness · Neuromuscular junction · Extensor digitorum longus muscle · BUF/Mna rat · Thymoma

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## Introduction

Much attention has been paid to the relationship between the thymic abnormality and muscle diseases [22]. For example, human myasthenia gravis [13, 33] and murine muscular dystrophy [3, 14] are closely related with various thymic abnormalities. BUF/Mna rats spontaneously develop thymomas in an autosomal dominant manner [27] and exhibit an exacerbated muscle fatigability and weakness during aging [25, 26]. The thymomas, histologically resembling human thymomas, start to develop from about 9 months after birth in males, and the incidence is 100% after 18 months of age [10]. Based on electrophysiological studies, Kato and Watanabe suggested that the motor dysfunction resulted mainly from some postsynaptic change at the site of neuromuscular junctions (NMJs) of fast twitch-type muscles [16, 17]. Furthermore, it has been reported recently that autoantibodies against ryanodine receptors, which function as calcium-release channels in the skeletal muscles, but not against acetylcholine receptors, are detected in the serum of this rat strain [15]. Thus, this rat strain has been recognized as a suitable rat model for human myasthenia gravis or related myophathies.

Previous ultrastructural studies of extraocular muscles and other fast twitch-type muscles in the aged rats by means of transmission electron microscopy (TEM) [25, 26] have shown the elongation and ramification of the postsynaptic membrane of the motor endplates, and moderate degeneration of the muscle fibers with lipid droplets and detached and distorted myofibrils. However, there has been little information on the three-dimensional organization of NMJs, particularly subneural apparatuses (SNAs), in any of the myasthenic muscle fibers both in humans and animal models at the fine structural level. For better understanding of the morphological changes of SNAs in the NMJs, it is effective to use scanning electron microscopy (SEM) after removing connective tissues [4, 6, 19].

The purpose of the present study was, therefore, to examine the ultrastructural changes of NMJs in the

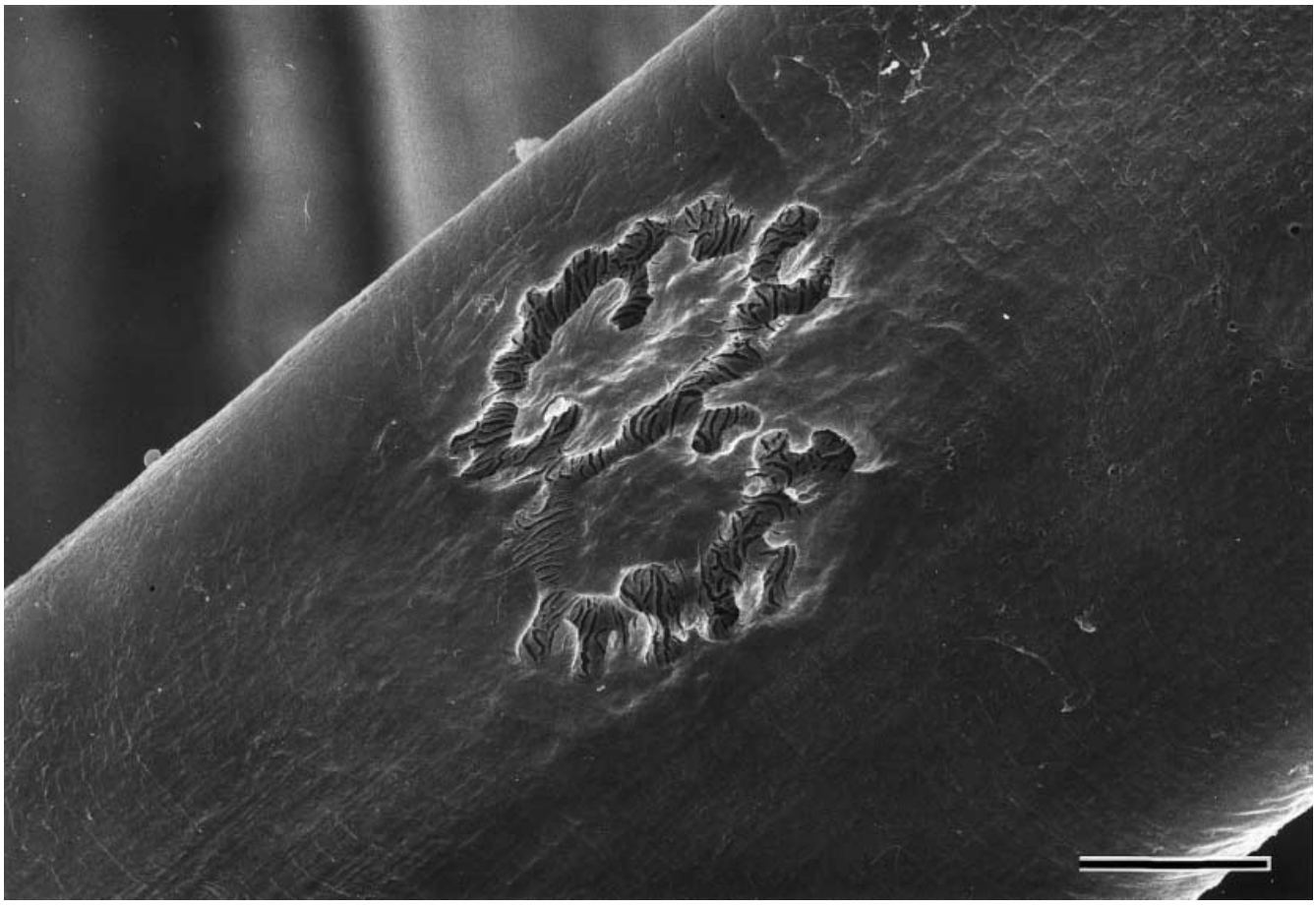


**Fig. 1** Scanning electron micrograph of a subneural apparatus (SNA) in the extensor digitorum longus muscle of a 4-month-old BUF/Mna rat. Note labyrinthine synaptic gutters containing numerous slit-like junctional folds. ×2500 (*scale bar* 10 μm)

extensor digitorum longus muscle, a typical fast twitch-type muscle, of aged male BUF/Mna rats at a three-dimensional level by means of SEM and TEM. The possible sites and mechanisms of the muscle dysfunction are discussed in comparison with morphological changes of NMJs following normal aging and the progress of diseases, such as human myasthenia gravis and murine muscular dystrophy which primarily cause muscle degeneration.

## Materials and methods

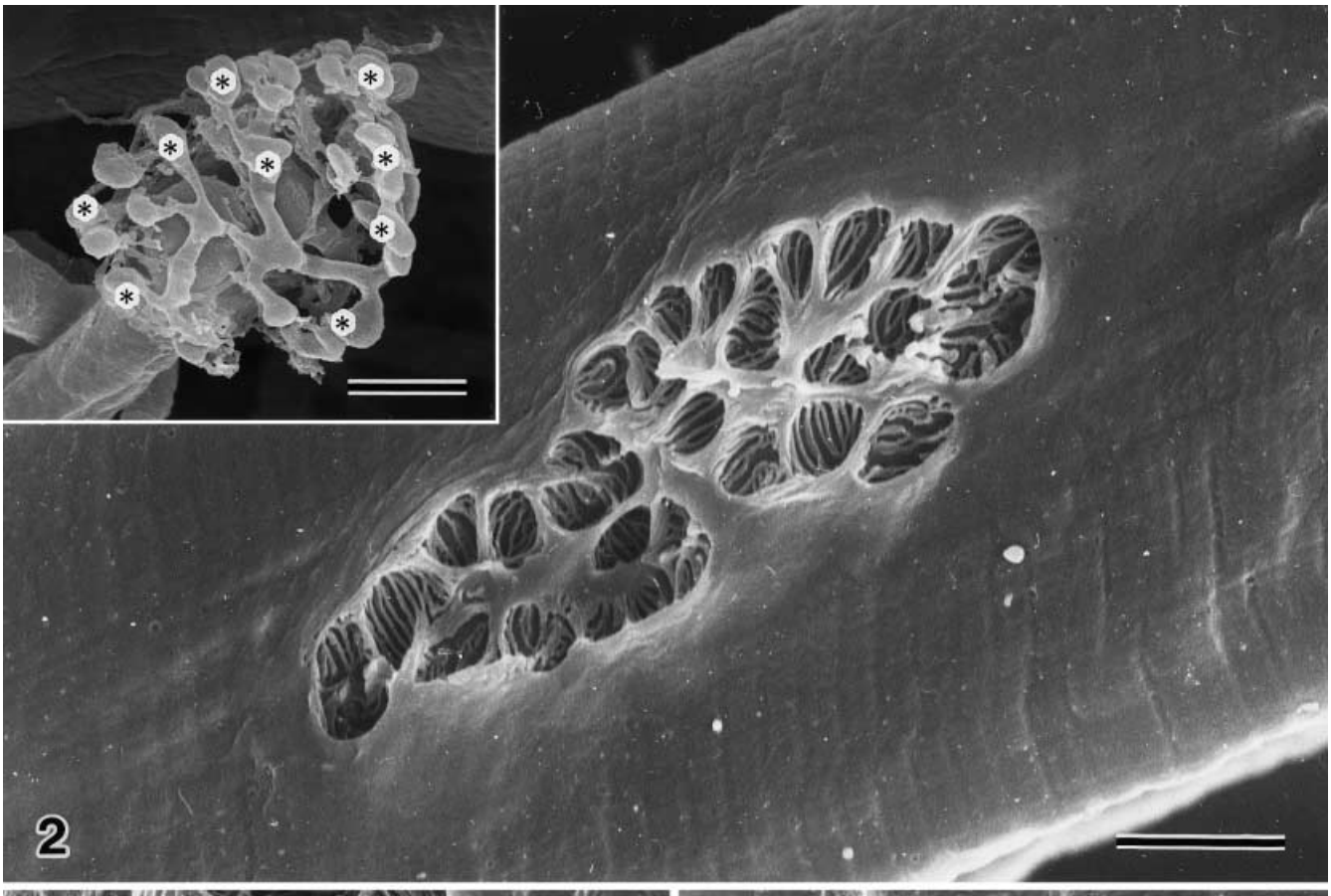
Three 4-month-old and five 22- to 25-month-old male BUF/Mna rats were used in this study. They were housed at a constant temperature (24°C) under a 12 h/12 h light/dark cycle and given food and water ad libitum. The following experiments were conducted in accordance with the legislation of Laboratory Animal Research Center for Animal Experimentation at Kumamoto University School of Medicine.

After the animals were anaesthetized via an intra-abdominal injection of 50 mg/kg pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, Ill.), the extensor digitorum longus muscles were exposed and fixed in situ with 4% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3 for 5 min. The muscles were dissected out, cut into small strips and further fixed with the same fixing solution for about 2 h. They were washed several times in the buffer and post-fixed with 2% unbuffered osmium tetroxide for about 30 min. For SEM observation, the specimens were treated with an HCl-hydrolysis procedure [4] to remove intramuscular connective tissues. After drying by the critical point method and sputter-coating with platinum, they were examined in a Hitachi S-800 scanning electron microscope. In some instances, the fixed specimens were block-stained with 3% aqueous uranyl acetate for 2 h and embedded in Epon-epoxy resin after dehydration through an ethanol series. Ultrathin sections were cut with a Porter-Blum ultramicrotome, doubly stained with uranyl acetate and lead citrate, and then examined with a Hitachi HU-12A transmission electron microscope.

## Results

In SEM observations of NMJs in the extensor digitorum longus muscle, SNAs of young-adult (4-month-old) male BUF/Mna rats consisted of complex labyrinthine synaptic gutters, about 2-μm wide, containing numerous slit-like junctional folds 0.1–0.2 μm in width and 1.5 μm in the maximum length (Fig. 1). The SNAs in young-adult BUF/Mna rats were almost similar in three-dimensional structure to those in normal young Wistar rats [29]. Muscle fibers were constantly 30–50 μm in diameter.

In aged (22- to 25-month-old) BUF/Mna rats, muscle fibers varied in size, ranging 10–50 μm in diameter. The small muscle fibers appeared to be in the course of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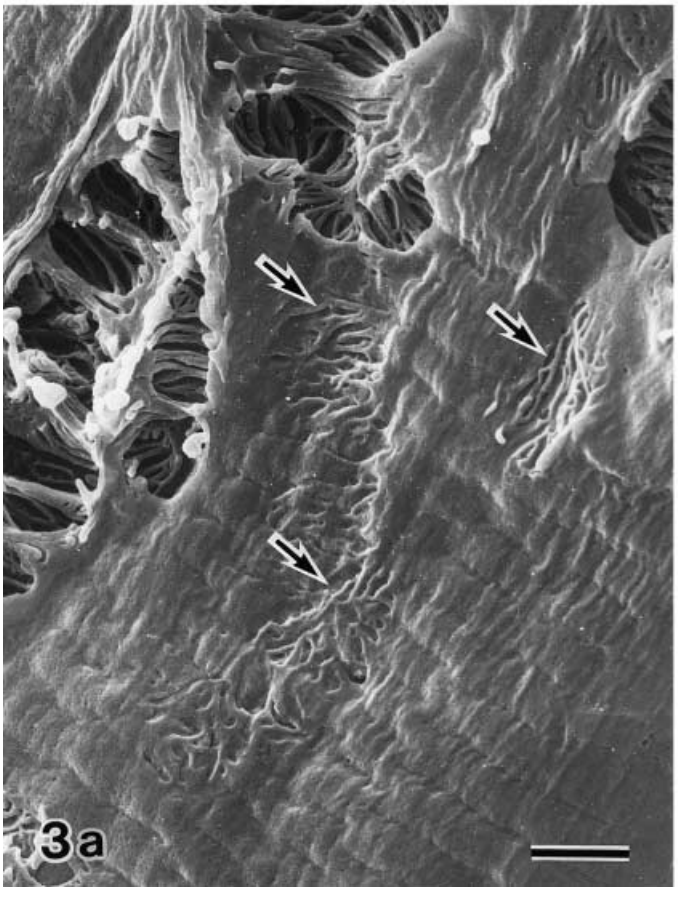
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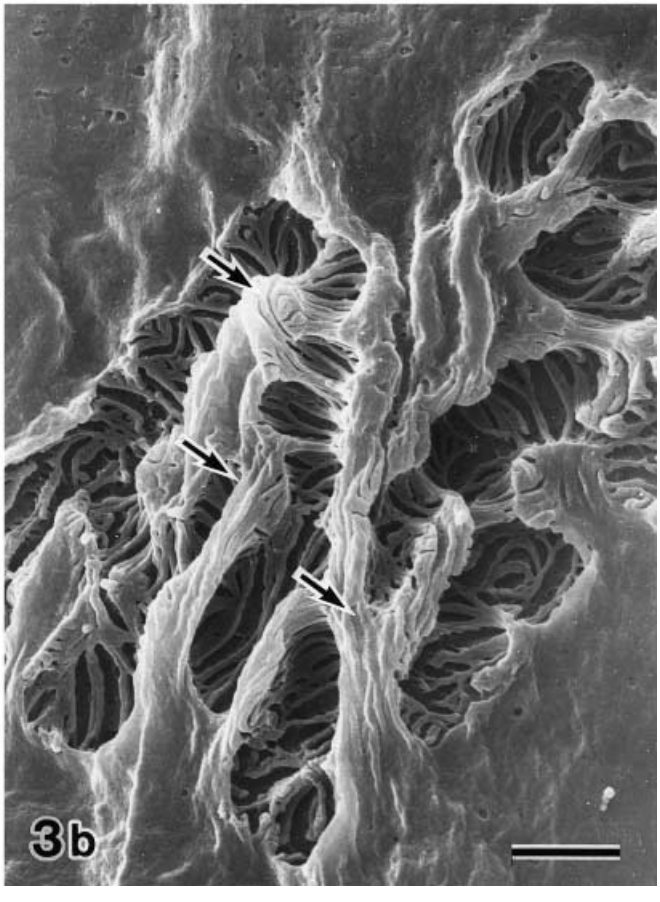
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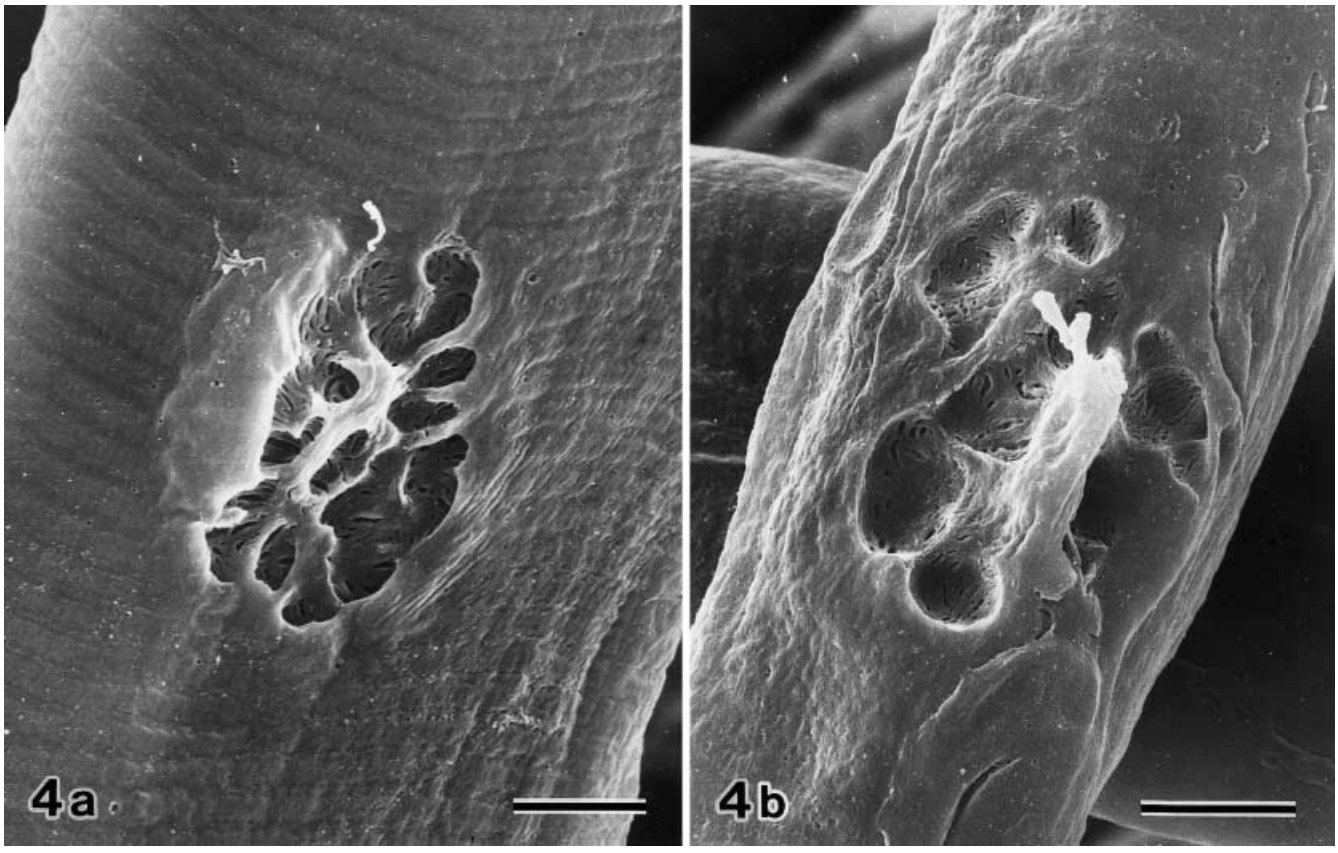



**Fig. 4** Scanning electron micrographs of subneural apparatuses (SNAs) of a 22-month-old BUF/Mna rat. **a** An SNA consisting of cup-like depressions with a small number of both pit-like and short slit-like junctional folds on a medium-sized muscle fiber about 30 μm in diameter. ×3400 (*scale bar* 5 μm). **b** An SNA consisting of a small number of cup-like depressions with some junctional folds on a small muscle fiber about 15 μm in diameter. ×3900 (*scale bar* 5 μm)

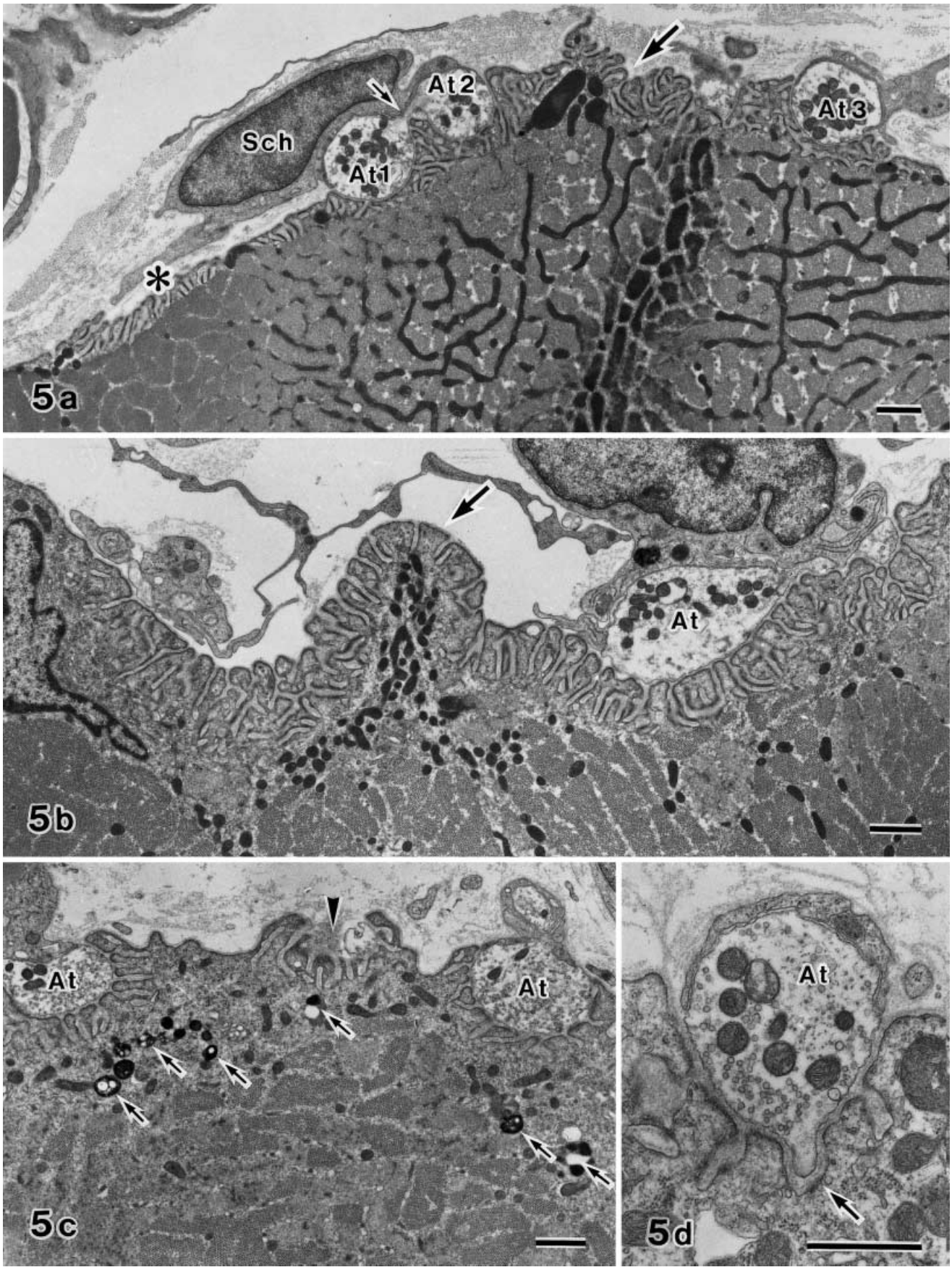
regeneration. In this study, a total of 44 SNAs from five aged rats were examined. The SNAs mainly consisted of a group of cup-like depressions (Fig. 2), differing from the complex labyrinthine gutters observed in the young muscles. Correspondingly, the nerve endings of aged NMJs were characterized by numerous terminal buttons or terminal varicosities 2–5 μm in diameter (Fig. 2, inset).

◀ **Fig. 2** Scanning electron micrograph of a subneural apparatus (SNA) on a large muscle fiber in the extensor digitorum longus muscle of a 22-month-old BUF/Mna rat. Note that the SNA consists of a number of cup-like depressions with slit-like junctional folds. ×4400 (*scale bar* 5 μm). *Inset* A nerve ending observed from the side facing the apparatus. Note numerous small protrusions (*asterisks*) of the terminal axon which are considered to correspond to individual depressions. ×3000 (*scale bar* 5 μm)

**Fig. 3** Scanning electron micrographs of subneural apparatuses (SNAs) on large muscle fibers of a 22-month-old BUF/Mna rat. **a** In addition to cup-like depressions with numerous slit-like junctional folds, numerous slit-like folds (*arrows*) are observed on the flattened sarcolemma outside the group of depressions. ×6200 (*scale bar* 2 μm). **b** Slit-like folds (*arrows*) are often observed on the crests or the protruded sarcoplasm between neighboring depressions. ×6700 (*scale bar* 2 μm)

At least two types of NMJs in the aged rats were distinguished based on the differences in the diameter of muscle fibers and organization of SNAs including the number of depressions and the developmental degree of junctional folds. One type of SNAs was observed in relatively large muscle fibers, 30–50 μm in diameter (32 instances of 44 SNAs), and consisted of a large number (more than 20) of cup-like depressions 2–5 μm in diameter (Fig. 2). Individual depressions predominantly contained slit-like junctional folds which were wider (0.3- to 0.5-μm wide) than those of young rats (Fig. 1). In eight SNAs on these muscle fibers, shallow gutters with shallow slits existed on the flat sarcoplasm outside the clusters of cup-like depressions (Fig. 3a). Moreover, slit-like folds were often found on the protruded junctional sarcoplasm between neighboring depressions (Fig. 3b). The other type of SNAs consisted of winding gutters or a number of (about ten) round depressions 2–5 μm in diameter. These depressions contained both slit-like and pit-like or elongated oval (0.1-μm wide and 0.1- to 0.5-μm long) junctional folds. These SNAs were observed on medium-sized muscle fibers (20–30 μm in diameter: five instances) (Fig. 4a) and on small muscle fibers (10–20 μm in diameter: seven instances) (Fig. 4b).

In TEM observation of NMJs in the aged BUF/Mna rats, nerve endings in contact with the depressions of plasma membrane of muscle fibers appeared to be intact (Fig. 5). The terminal axons had a great number of syn-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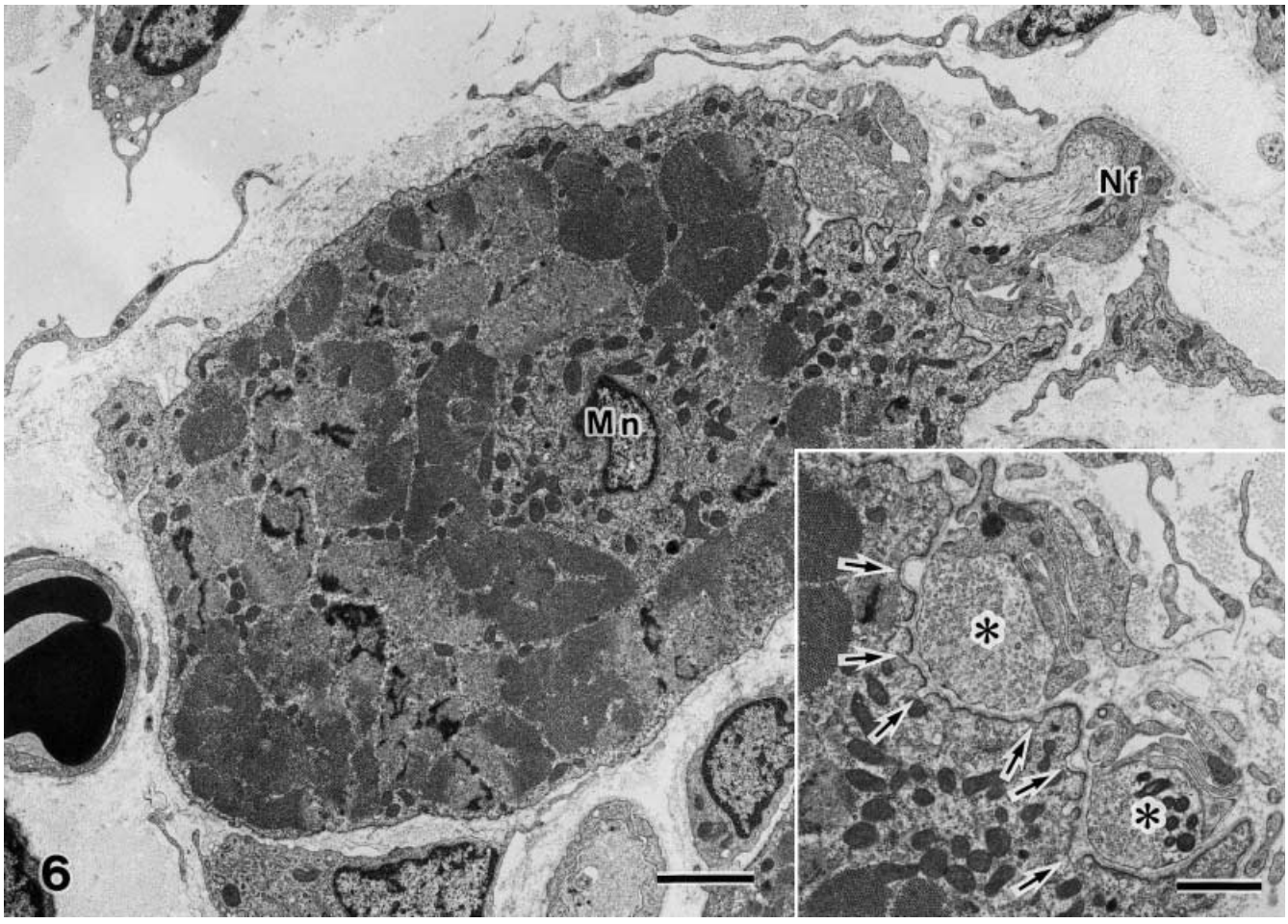


**Fig. 6** Transmission electron micrographs of a small muscle fiber in the extensor digitorum longus muscle of the 25-month-old BUF/Mna rat. The small fiber, having a centrally located nucleus (*Mn*), is innervated by a nerve fiber (*Nf*). ×6600 (scale bar 2 μm). *Inset* two terminal axons (*asterisks*) in shallow indentations contain numerous synaptic vesicles, but secondary synaptic clefts are few in number and incipient (*arrows*). ×11000 (scale bar 1 μm)

◀ **Fig. 5** Transmission electron micrographs of neuromuscular junctions (NMJs) in large muscle fibers of the extensor digitorum longus muscle in a 22-month-old BUF/Mna rat. **a** Terminal axons (at *1, 2, 3*) containing numerous synaptic vesicles and mitochondria lay on the shallow depressions. Two terminal axons (at *1, 2*) are connected to each other by a thin axon (*small arrow*). Note an expanse of junctional folds lacking terminal axons on the sarcoplasmic protrusion between neighboring terminal axons (*large arrow*) and on the remote sarcoplasm from the terminal axon (*asterisk*). *Sch* Schwann cell. ×7800 (*scale bar* 1 μm). **b** A protruded junctional sarcoplasm (*arrow*) has secondary synaptic clefts but lacks terminal axons. *At* terminal axon. ×9500 (*scale bar* 1 μm). **c** Numerous lysosome-like granules (*arrows*) are observed in junctional sarcoplasm. Note that a primary cleft (*arrowhead*) has no longer a competent terminal axon. *At* terminal axon. ×8800 (*scale bar* 1 μm). **d** A small and short process from a terminal axon (*At*) protrudes into a wide junctional fold (*arrow*). ×21000 (*scale bar* 1 μm)

aptic vesicles approximately 50 nm in diameter and a number of mitochondria. In the postsynaptic membrane of large muscle fibers (30–50 μm in diameter), secondary synaptic clefts devoid of any overlying nerve endings were often seen on the protruded sarcoplasm between neighboring primary synaptic clefts (Fig. 5a, b), or on the remote flat sarcoplasm from the primary clefts (Fig. 5a). The postsynaptic sarcoplasm often contained numerous electron-dense lysosomal granules (Fig. 5c). In these NMJs, terminal axons partially degenerated and some primary clefts lacked them. Moreover, in some NMJs, small budding-like short processes of terminal axons (0.2–0.4 μm in diameter and 0.2–0.5 μm in length) protruded into the wide secondary clefts (Fig. 5d). In small and medium-sized muscle fibers (10–30 μm in diameter), however, the postsynaptic structures were rather simple. The postsynaptic membrane consisted of a few or a small number of poorly developed junctional folds (Fig. 6 and inset), possibly indicating incipient junctional folds. These small muscle fibers often have centrally located nuclei.

## Discussion

The aim of the present study was to provide new information using three-dimensional observations on NMJs in

the thymoma BUF/Mna rats that suffer from muscle dysfunction during aging. In addition to the previous findings [26], such as size variation of muscle fibers, we found several interesting features of NMJs in the thymoma rats, in that they have some similarity to other myopathies or normal aging processes. When comparing with those in young adult controls, the SNAs in the aged BUF/Mna rats were characterized by: (1) a group of cup-like depressions with very wide slit-like junctional folds in relatively large muscle fibers (the major type), (2) the presence of slit-like folds on the flat sarcoplasm outside the cup-like depressions or on the protruded sarcoplasm, and (3) winding gutters or a small number of round depressions with poorly developed synaptic folds in small and medium-sized muscle fibers (the minor type). These features were also confirmed by TEM observations.

In general, SNAs in normal adult mammalian skeletal muscles are characterized by labyrinthine gutters with numerous slit-like junctional folds [4, 5, 6, 11, 19, 24, 28, 29]. The group of cup-like depressions seen in the aged BUF/Mna rats, however, are rarely found in young rats. Interestingly, such similar structural changes in SNAs have been observed by SEM in regenerating muscle fibers after necrosis in the muscular dystrophic (*dy*, *dy*$^{2J}$ and *mdx*) mice [6, 19, 20]. The structural changes of dystrophic SNAs are considered to be independent of the direct response to dystrophic deficiency that causes muscle degeneration [23]. Thus, the gutter-into-cup transformation in SNAs may take place as a result of degeneration and regeneration of the muscle fibers following aging [2, 31], probably reflecting adequate structural remodeling for nerve-to-muscle signal transduction. The slit-like folds found on the flat sarcoplasm outside the depressions and on the protruded sarcoplasm seem to correspond to the expanses of junctional folds lacking terminal axons observed by TEM in this study (Fig. 5a, b) and in previous studies on human myasthenia gravis [32]. The occurrence of these slits may indicate that vestigial junctional folds persist for a long period after spontaneous denervation, probably depending on the existence of a cytoskeletal network underlying the plasma membrane of their crests [8, 12]. In other words, these postsynaptic abnormalities may take place as a result of terminal sprouting and remodeling after retraction of nerve endings, which is caused by partial degeneration of muscle fibers. In addition, we often found NMJs containing numerous lysosome-like granules in junctional sarcoplasm (Fig. 5c), probably representing a degenerative figure [7]. In this context, this seems to support that the degeneration of junctional sarcoplasm induces the retraction of nerve endings. All these structural changes were found mostly on relatively large muscle fibers, and this was the major type of SNAs in the aged rats (approximately 70% of all the examined NMJs).

However, the SNAs on relatively small muscle fibers of aged thymoma rats consisted of a small number of cup-like depressions with poorly developed synaptic folds. The junctional folds have not developed enough. It can be suggested that these SNAs are newly formed on regenerating muscle fibers after their necrosis and are in the course of maturation. In fact, the small muscle fibers in the aged BUF/Mna rats often have centrally located nuclei (Fig. 6), which may represent a sign of muscle regeneration [7]. Such similar immature structures of SNAs in regenerating muscle fibers with centrally located nuclei were also observed in the dystrophic (*mdx*, *dy*$^{2J}$ and *dy*) mice and became more obvious with age [6, 21, 23, 34]. Alternatively, the apparatus containing sparse, narrow, slit-like and pit-like junctional folds may just happen to be those on the slow twitch-type fibers scattered in the extensor digitorum longus muscle [29] rather than on the regenerating fast twitch-type fibers. However, our TEM observation revealed that short and thin processes from the terminal axons often protrude into wide junctional folds in the aged BUF/Mna rats. The existence of these structures suggests that the terminal axons may exhibit intact presynaptic transmitter release and recycling of synaptic vesicles, while the postsynaptic membrane may indicate a degenerating sign. This is supported by previous findings in the electrophysiological examination of this thymoma rats [15, 16]. Similar structural changes were observed in small subneural sarcoplasmic pits (0.5–1.0 μm in diameter) of the 4-month-old dystrophic (dy) mouse [6].

The similarities in these various structural changes found in the aged BUF/Mna rats to those found in other myopathies or normal aging processes may indicate that the muscle turnover mechanism in the aged rats is preserved. Thus, we would suggest that both the slowly progressing muscle atrophy [1] and age-dependent turnover of muscle fibers [11, 30] coexist in the aged thymoma rats. The mechanism of how the remodeling of NMJs seen in BUF/Mna rats is induced, however, still remains to be determined. Iwasa et al. [15] have hypothesized that the functional defect in excitation–contraction coupling of the hind leg muscles is caused by autoimmune anti-ryanodine receptor antibodies raised against thymic epithelial cells in BUF/Mna rats. However, there is a discrepancy in the time of occurrence between the fine structural changes of NMJs and the anti-ryanodine receptor autoantibodies which are detected at as early as 3 months of age [15]. Furthermore, the occurrence of the autoantibodies does not appear to correlate directly with the thymoma development, since thymomas in male BUF/Mna rats start to develop at about 9 months of age [10]. Since the muscle atrophy develops slowly [1], it may take such a long period until the fine structural changes of SNAs become evident. Alternatively, it has been known that this strain of rats complicates abnormal lipid metabolism and hypoproteinemia with creatinuria, which could be a possible cause of muscle weakness and fatigability and variable changes in muscle fibers [18]. In addition, the thymomas usually reach more than 20 g [9] and polycythemia is often seen probably due to breathing problem caused by the tumor mass (unpublished observation). Therefore, it is even likely that the metabolic disturbance accelerated by such various complications with age causes the muscle degeneration and therefore induces the remodeling of NMJs in the hind leg muscles of these rats.

In conclusion, from the present findings and previous observations, we confirm that the NMJs in aged BUF/Mna rats perform similar structural changes to those in normally aged rats and dystrophic (*dy*) mice. It is suggested that both the slowly progressing muscle atrophy and age-dependent turnover of muscle fibers may occur in the aged BUF/Mna rats. With respect to the relationship of the thymoma development to the structural changes of NMJs, therefore, we suggest that the progress of this disease does not directly influence the structural changes of aged NMJs in this rat strain.

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## References

1. Amo H, Saito M, Nagao S, Yagiu S, Takahashi H, Morita H, Morita TM, Matsuyama M (1997) Genetic regulation of slowly progressing mild muscle atrophy in fast-twitch muscles of BUF/Mna rats. Muscle Nerve 20:1258–1263
2. Cardasis CA (1983) Ultrastructural evidence of continued reorganization at the aging (11–26 months) rat soleus neuromuscular junction. Anat Rec 207:399–415
3. de Kretser TA, Livett BG (1976) Evidence of a thymic abnormality in murine muscular dystrophy. Nature 263:682–684
4. Desaki J, Uehara Y (1981) The overall morphology of neuromuscular junctions as revealed by scanning electron microscopy. J Neurocytol 10:101–110
5. Desaki J, Uehara Y (1987) Formation and maturation of subneural apparatuses at neuromuscular junctions in postnatal rats: a scanning and transmission electron microscopical study. Dev Biol 119:390–401
6. Desaki J, Matsuda S, Sakanaka M (1995) Morphological changes of neuromuscular junctions in the dystrophic (dy) mouse: a scanning and transmission electron microscopic study. J Electron Microsc (Tokyo) 44:59–65
7. Dubowitz V (1985) Muscle biopsy: a practical approach, 2nd edn. Bailliere Tindall, London
8. Ellisman MH, Rash JE, Staehelin LA, Porter KR (1976) Studies of excitable membranes. II. A comparison of specializations at neuromuscular junctions and nonjunctional sarcolemmas of mammalian fast and slow twitch muscle fibers. J Cell Biol 68:752–774
9. Ezaki T, Kawatsu R, Matsuno K, Kotani M (1990) Characterization of intrathymic and extrathymic T cell development in spontaneous thymoma Buffalo/Mna rats. Thymus 16:67–87
10. Ezaki T, Fujii H, Matsuno K, Kawatsu R, Kotani M (1992) Oestrogen retards the development of spontaneous thymomas in BUF/Mna rats. Virchows Arch 421:505–511
11. Fahim MA, Holley JA, Robbins N (1983) Scanning and light microscopic study of age changes at a neuromuscular junction in the mouse. J Neurocytol 12:13–25
12. Hirokawa N, Heuser JE (1982) Internal and external differentiations of the postsynaptic membrane at the neuromuscular junction. J Neurocytol 11:487–510
13. Hohlfeld R (1990) Myasthenia gravis and thymoma: paraneoplastic failure of neuromuscular transmission (editorial; comment). Lab Invest 62:241–243
14. Horrobin DF, Morgan RO, Karmali RA, Ally AI, Manku MS, Karmazyn M, Cunnane SC (1979) Thymic changes in muscular dystrophy and evidence for an abnormality related to prostaglandin synthesis or action. Ann NY Acad Sci 317:534–549
15. Iwasa K, Komai K, Takamori M (1998) Spontaneous thymoma rat as a model for myasthenic weakness caused by anti-ryanodine receptor antibodies. Muscle Nerve 21:1655–1660
16. Kato F, Watanabe M (1982) Motor dysfunction in spontaneous thymoma rats, Buffalo/Mna. J Pharmacobiodyn 5:727–733
17. Kato F, Watanabe M (1982) Motor dysfunction in thymoma rats: comparison between fast and slow muscles. J Pharmacobiodyn 5:1005–1011
18. Kato F, Watanabe M (1983) Biochemical study on spontaneous thymoma rats with motor dysfunction. J Pharmacobiodyn 6: 275–279
19. Kitaoka K, Matsuda Y, Desaki J, Oki S, Nagano Y, Shibata T (1997) Topographic comparison of subneural apparatuses at neuromuscular junctions in normal and dystrophic (mdx) mice: a scanning electron microscope study. J Electron Microsc (Tokyo) 46:193–197
20 Labrecque C, Tremblay JP, Fahim MA (1988) Scanning electron microscopic study of the neuromuscular junction of dystrophic mice. Exp Neurol 100:51–64
21. Law PK, Saito A, Fleischer S (1983) Ultrastructural changes in muscle and motor end-plate of the dystrophic mouse. Exp Neurol 80:361–382
22. Levinson AI, Wheatley LM (1996) The thymus and the pathogenesis of myasthenia gravis. Clin Immunol Immunopathol 78:1–5
23. Lyons PR, Slater CR (1991) Structure and function of the neuromuscular junction in young adult mdx mice. J Neurocytol 20:969–981
24. Matsuda Y, Oki S, Kitaoka K, Nagano Y, Nojima M, Desaki J (1988) Scanning electron microscopic study of denervated and reinnervated neuromuscular junction. Muscle Nerve 11: 1266–1271
25. Matsuyama M, Ito H, Masuyama T, Nakamura T, Iida M (1978) Spontaneous muscle weakness in high thymoma Buffalo/Mna rats. Nagoya Med J 23:93–98
26. Matsuyama M, Amo H, Takayanagi T, Iida M (1981) Motor dysfunction and muscular abnormalities in rats of high thymoma BUF/Mna strain. In: Satoyoshi E (ed) Myasthenia gravis. Pathogenesis and treatment. University of Tokyo Press, Tokyo, pp 81–92
27. Matsuyama M, Yamada C, Hiai H (1986) A single dominant susceptible gene determines spontaneous development of thymoma in BUF/Mna rat. Jpn J Cancer Res 77:1066–1068
28. Ogata T, Yamasaki Y (1985) The three-dimensional structure of motor endplates in different fiber types of rat intercostal muscle. A scanning electron-microscopic study. Cell Tissue Res 241:465–472
29. Oki S, Matsuda Y, Kitaoka K, Nagano Y, Nojima M, Desaki J (1990) Scanning electron microscope study of neuromuscular junctions in different muscle fiber types in the zebra finch and rat. Arch Histol Cytol 53:327–332
30. Oki S, Desaki J, Ezaki T, Matsuda Y (1999) Aged neuromuscular junctions in the extensor digitorum longus muscle of the rat as revealed by scanning electron microscopy (letter). J Electron Microsc (Tokyo) 48:297–300
31. Robbins N, Fahim MA (1985) Progression of age changes in mature mouse motor nerve terminals and its relation to locomotor activity. J Neurocytol 14:1019–1036
32. Santa T, Engel AG, Lambert EH (1972) Histometric study of neuromuscular junction ultrastructure. I. Myasthenia gravis. Neurology 22:71–82
33. Simpson JA (1960) Myasthenia gravis: a new hypothesis. Scot Med J 5:419–436
34. Torres LF, Duchen LW (1987) The mutant mdx: inherited myopathy in the mouse. Morphological studies of nerves, muscles and end-plates. Brain 110:269–299