Original article

Folia Neuropathologica

Motor endplate remodeling in some cases with congenital myasthenic syndrome

Anna Fidziańska, Zofia Glinka

Neuromuscular Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Folia Neuropathol 2010; 48 (3): 200-205

Abstract

The architecture of motor endplates in three cases with congenital myasthenic syndrome (CMS) was compared with ultrastructure of the normal control neuromuscular junction (NMJ). The remodeling of postsynaptic region was observed in all three individuals. The most conspicuous abnormalities seen in the slow channel syndrome was the vacuolization and disorganization of secondary synaptic clefts which extended for beyoned the border of NMJ. Degenerated postsynaptic nuclei and junctional sarcoplasm were an additional feature of presented syndromes. The quite different feature of NMJ was observed in the DOK-7 deficient syndrome. The appearance of small, pale terminal axons, poorly developed postsynaptic membrane with the sparse secondary synaptic clefts and degenerated postsynaptic nuclei suggested impairment of postsynaptic region maturation. The conjunction of postsynaptic membrane paucity and its degeneration was a specific structural feature observed in the third syndrome with no established genetic defects.

Key words: motor endplates, congenital myasthenic syndrome, neuromuscular junction.

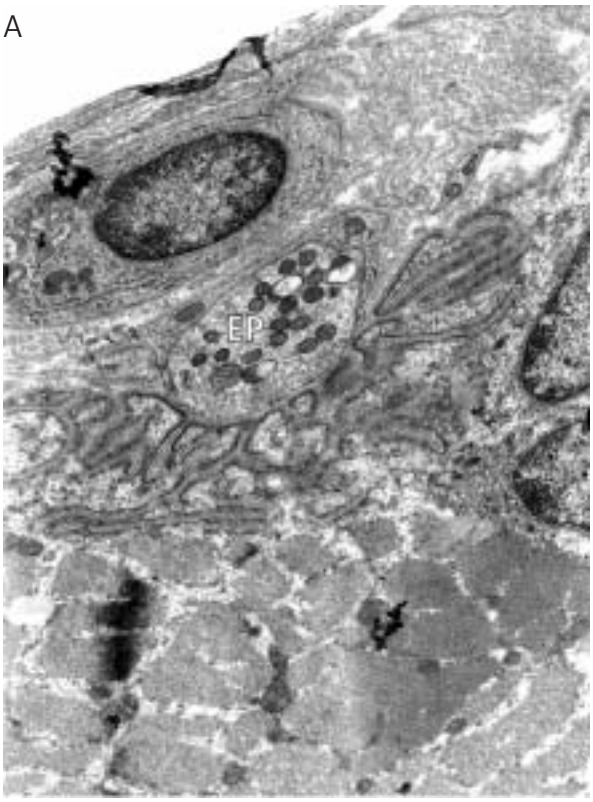
Introduction

The motor endplate is highly specialized in morphology and molecular composition connection between nerve and muscle cell which is able to transduce signals from the nerve terminal to skeletal muscle fibre. The development and differentiation of the neuromuscular junction (NMJ) is a multisteps process requiring coordinated interaction between muscle cell and nerve terminal. The initial step in NMJ formation and differentiation require activity of numerous cytoplasmic proteins [11]. Genetic defects in presynaptic, synaptic and postsynaptic junction are known as congenital myasthenic syndromes (CMS) [2,6-8,13]. Truncated proteins or their loss affects the architecture and function of NMJ. Using electron microscopy, we analysed endplate architecture and described ultrastructural abnormalities that occur in the endplates of patients with congenital myasthenic syndromes.

Material and methods

The structure of motor endplates was analysed in three patients with disorder neuromuscular transmission. The first patient was a young woman with the slow channel syndrome and a mutation in AChR epsilon subunit [9]. The second patient was a boy with mutation in the DOK-7 gene (Engel *et al.* data not

Communicating author:
Anna Fidziańska, Neuromuscular Unit, Medical Centre, Polish Academy of Science, Pawińskiego 5, 02-106 Warsaw, Poland,
phone/fax: +48 22 658 45 01, e-mail: neurmyol@cmdik.pan.pl



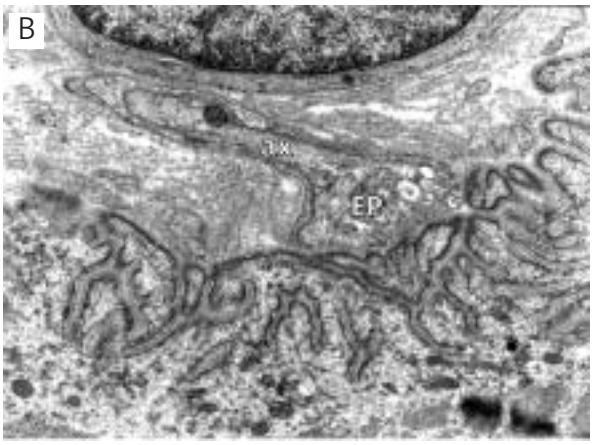


Fig. 1. A) Control motor endplate (EP) with numerous synaptic vesicles and numerous synaptic clefts. × 30 000; **B)** Normal motor endplate (EP) with terminal axon (ax) longitudinally oriented. × 36 000.

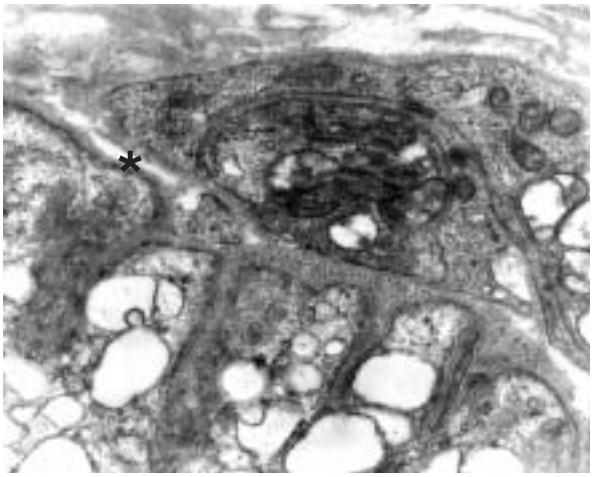


Fig. 2. Case 1. The significantly widened primary synaptic cleft (asterisk) with dense punctuate granules. × 45 000.

published) who had a similarly affected younger brother. The third patient was a man with undetermined genetic diagnosis (a mutation in the AChR gene was excluded) who had clinical and electrophysiological signs of CMS. The quadriceps femoris muscle of all patients was analysed. As a control motor endplates of unaffected quadriceps femoris muscles were investigated.

For electron microscopy specimens were fixed in the 1% glutaraldehyde in phosphate buffer and postfixed in 1% osmium tetroxide in the same buffer. Then they were dehydrated and embedded in Spurr resin. Thin sections double stained with uranyl acetate and lead citrate were examined with JEM electron microscope.

Results

The normal synapse contains three major structural elements: the presynaptic nerve terminal that is capped by a Schwann cell, the primary synaptic cleft occupied by basal lamina and secondary synaptic clefts formed by folds in the postsynaptic membrane (Fig. 1A). The hallmark of the nerve terminal is the appearance of the synaptic vesicles which are located precisely across synaptic cytoplasm. The synaptic space of 5.0 nm, called primary synaptic cleft, separates the terminal nerve and muscle cell plasma membrane. Plasma membrane of the muscle cell is lined with basal lamina which contains numerous proteins regulating synapse formation and activity. The secondary synaptic clefts in normal EP are well developed. They are numerous, long, closely packed and often branched (Fig. 1B). In the case with mutation in AChR epsilon subunit, the shape and size of the terminal nerve varied as did the extent of folding of the postsynaptic membranes. A few swelling mito-

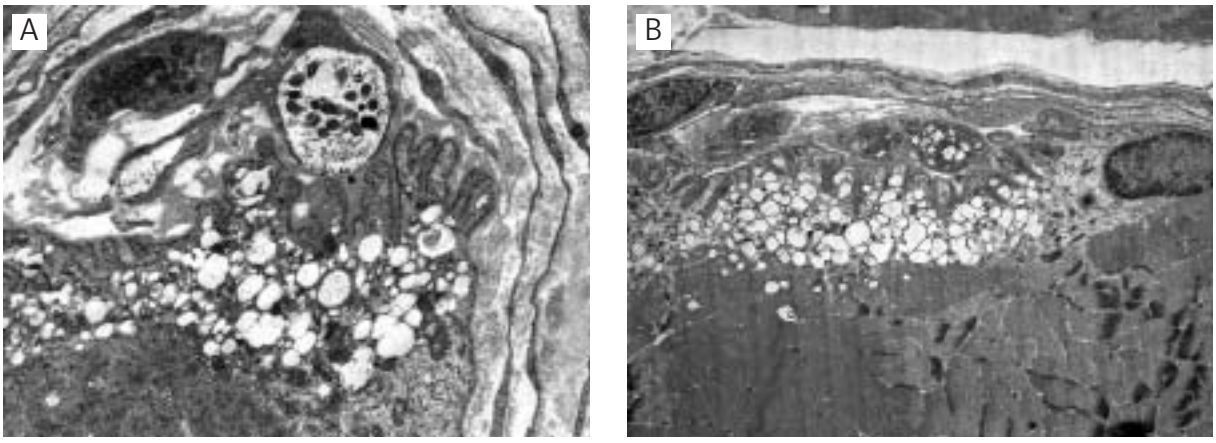


Fig. 3. Case 1. **A)** Degenerated and vacuolated postsynaptic junction. × 23 000; **B)** Vacuolated postsynaptic sarcoplasm. × 12 000.

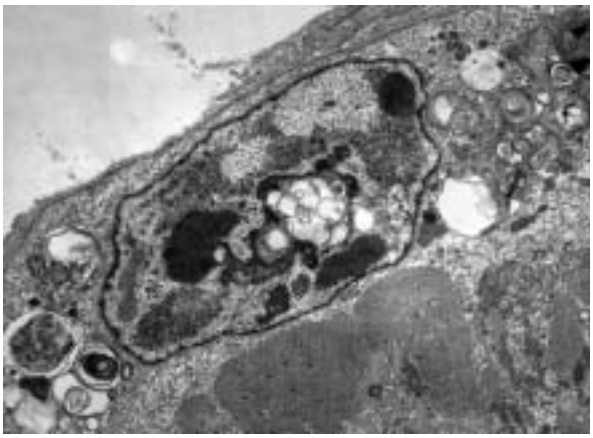


Fig. 4. Case 1. Degenerated postsynaptic nucleus. × 22 000.

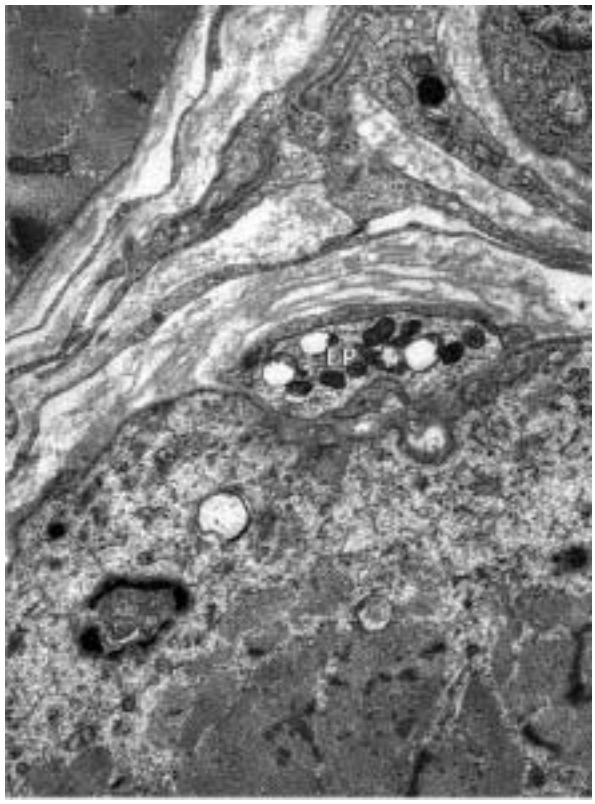


Fig. 5. Case 2. A small endplate (EP) with swelling mitochondria and widened primary cleft. × 22 000.

chondria were observed in endplates. The synaptic space was significantly widened with decreasing density of basal lamina as well as with the presence of numerous dense punctuate granules (Fig. 2). The abnormality of the postsynaptic area of NMJ was the destruction of postjunctional folds and the appearance of membrane-bounded vacuoles (Fig. 3A,B). The junctional sarcoplasm was filled with vacuole-like structures ranging in size from 0.05 to 1 µm. They are empty or filled with degenerated membranes. Postsynaptic nuclei showed different stages of degeneration (Fig. 4) including vacuolization, heterochromatin condensation. The appearance of various inclusions in nuclear matrix was frequently observed.

In the case with a mutation in the DOK 7 gene, the motor enplate revealed abnormality of pre- and postsynaptic regions compared to the architecture of normal NMJ. Terminal axons showed a small size, swelling mitochondria a decreased number of synaptic vesicles (Fig. 5). The primary synaptic defts were widened. The number of postsynaptic folds (secondary synaptic clefts) was significally reduced. The post-

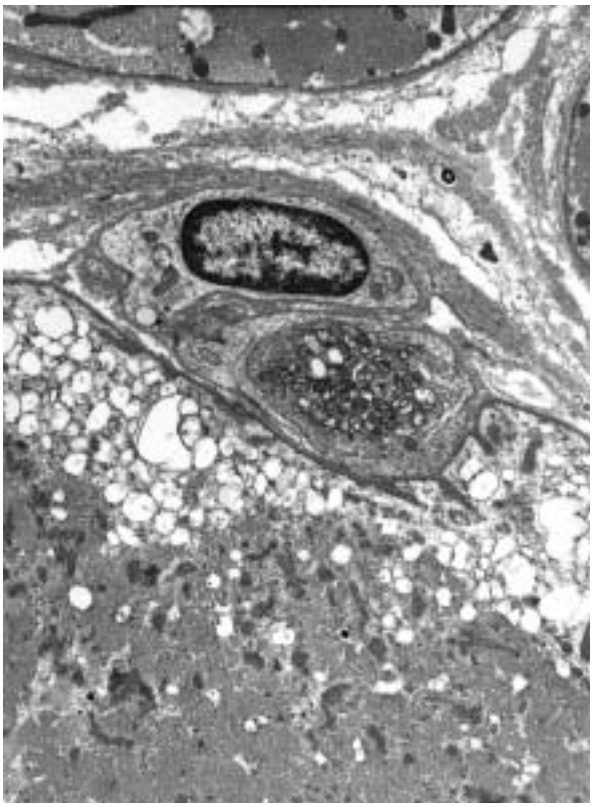


Fig. 6. Case 2. The significantly reduced postsynaptic folds. Vacuolated junctional sarcoplasm. × 18 000.

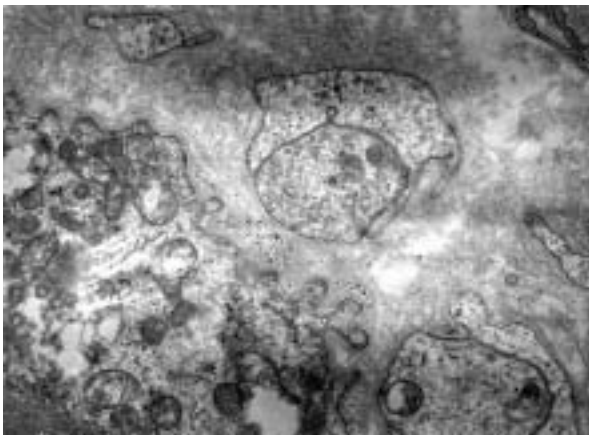


Fig. 7. Case 3. Extremely widened primary synaptic cleft. × 36 000.

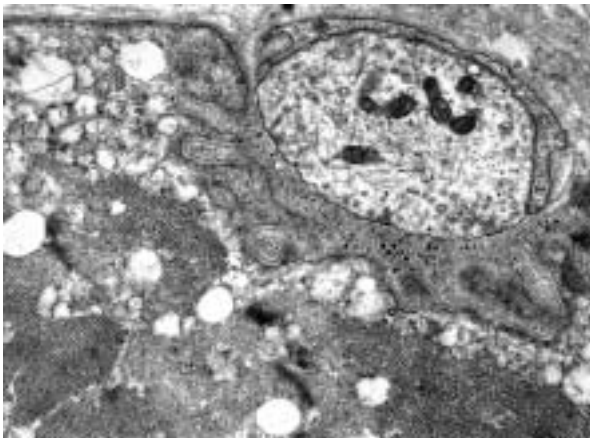


Fig. 8. Case 3. The paucity of secondary postsynaptic clefts and widened primary synaptic cleft. × 45 000.

synaptic membrane was straight, with few short foldings (Fig. 6). The junctional sarcoplasm showed degenerated mitochondria, membrane-bound structures (Fig. 6) and degenerating synaptic nuclei.

In the third case, electron microscopy analysis revealed small nerve terminals, extremely widened synaptic space (Fig. 7) reduced junctional folds (Fig. 8) and a very large destroyed postjunctional region. Postjunctional sarcoplasm created with tubular network showed numerous myelin structures and degenerated nuclei (Fig. 9A,B).

Discussion

In this report, we describe motor endplate remodeling in three cases with impaired neuromuscular transmission diagnosed as the congenital myasthenic syndrome (CMS). Ultrastructural analysis showed that in all three cases, the postjunctional region of NMJ underwent reorganization and deformation. Significant widening of the synaptic space with the appearance of dense, punctuate granules was observed in all affected cases. In contrast to these marked changes in the postsynaptic region, notable lack of degeneration of the nerve terminals was seen. Degeneration of postsynaptic junctions and their loss were observed in the case with mutation in epsilon unit of AChR gene as well as in the genetically undetermined case. The postsynaptic folds reorganization, destruction and vacuolization of sarcoplasmic components have been observed in both cases, although in the case with a mutation in epsilon AChR gene accumulation of vacuolar structures was the dominating feature. In addition, vacuolated mitochondria, myelin structures and autophagic vacuoles were frequently found.

Postsynaptic nuclei exhibited varied stages of degeneration in both investigated cases. Such reor-

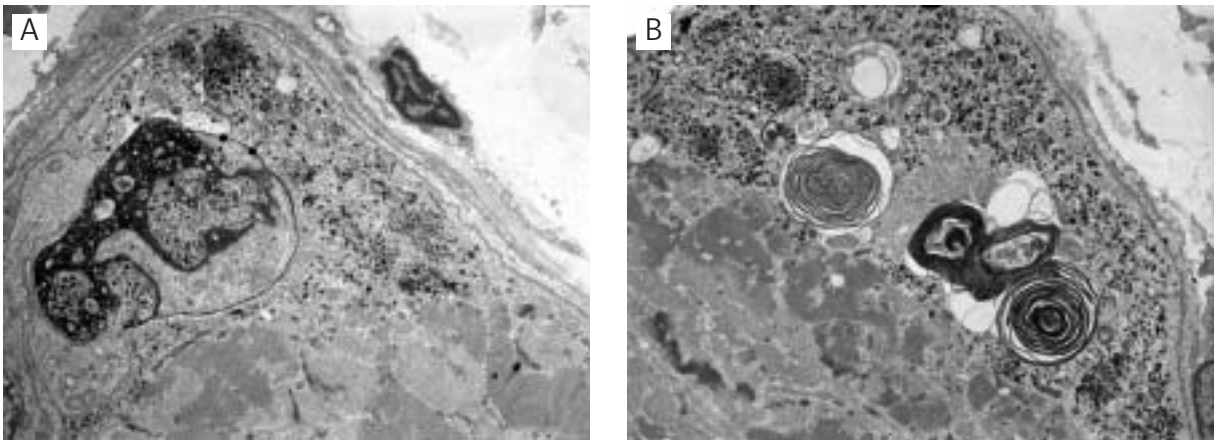


Fig. 9. Case 3. **A)** Degenerated postsynaptic nuclei. × 12 000; **B)** The junctional sarcoplasm with numerous myelin figures. × 12 000.

ganization and degeneration of postsynaptic region with destruction of postjunctional folds was previously reported in the acetylocholinesterase deficiency syndrome [5,9,10]. In the slow channel syndrome, the prolonged opening time of AChR ion channel results in an induced influx of calcium ions at the postsynaptic region [10]. Increased amount of calcium and another divalent cations activates cytoplasmic proteases which cause destruction of junctional folds and other postsynaptic elements [5,10]. The interesting motor endplate abnormality was observed in the case with mutation in DOK7 gene. In contrast to control EP, in which the secondary synaptic clefts are well developed, closely packed and often branched, in our case the postsynaptic regions of the NMJs were poorly developed, sparse, short and unbranched. In the same endplates, the postsynaptic membrane was abnormally straight with few short infoldings. The junctional sarcoplasm showed degenerated mitochondria, membrane-bound structures and degenerating synaptic nuclei. Such paucity of secondary synaptic clefts may suggest failure of postsynaptic function and maturity. DOK-7 protein is a cytoplasmic activator of muscle specific receptor tyrosine-kinase (MuSK). Both DOK-7 and MuSK are essential for specialization of the postsynaptic membrane [2,11]. Agrin, which is released from the nerve terminal, activates MuSK located in the postsynaptic membrane, which leads to precise localization and aggregation of acetylocholine receptors (AChR) through their association with the cytoplasmic anchoring protein-rapsyn [13]. An additional component, cytoplasmic molecule DOK-7, contributes to this pathway [1]. Recently, mutation in the newly identified endplate protein DOK-7 has been shown to affect AChR clustering and created a new type of congenital myasthenic syndrome with postsynaptic immaturity [1,11,12]. Our observations indicate that remodeling or significant destruction of the postsynaptic region of motor endplate seen at the ultrastructural level may be an important ultrastructural marker of congenital myasthenic syndromes.

References

1. Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, Newsom-Davis J, Burke G, Fawcett P, Motomura M, Müller JS, Lochmüller H, Slater C, Vincent A, Yamanashi Y. DOK-7 mutations underlie a neuromuscular junction synaptopathy. Science 2006; 313: 1975-1978.
2. Beeson D, Webster R, Cossins J, Lashley D, Spearman H, Maxwell S, Slater CR, Newsom-Davis J, Palace J, Vincent A. Congenital myasthenic syndromes and the formation of neuromuscular junction. Ann N Y Acad Sci 2008; 1132: 99-103.
3. Engel AG. The therapy of congenital myasthenic syndromes. Neurotherapeutics 2007; 4: 252-257.
4. Engel AG, Lambert EH, Gomez MR. A new myasthenic syndrome with endplate acetylocholinesterase deficiency, small nerve terminal and reduced acetylocholine release. Ann Neurol 1977; 1: 315-330.
5. Engel AG, Lambert EH, Mulder DM, Torres CF, Sahashi K, Bertorini TE, Whitaker JN. A newly recognized congenital myasthenic syndrome attributed to a prolonged open time of the acetylocholine-induced ion channel. Ann Neurol 1982; 11: 553-569.
6. Engel AG, Ohno K, Shen XM, Sine SM. Congenital myasthenic syndromes multiple molecular targets at the neuromuscular junction. Ann N Y Acad Sci 2003; 998: 138-160.

7. Engel AG, Ohno K, Sine SM. Congenital myasthenic syndromes: diverse array of molecular targets. J Neurocytol 2003; 32: 1017-1030.
8. Engel AG, Shen XM, Selcen D, Sine SM. Further observations in congenital myasthenic syndromes. Ann N Y Acad Sci 2008; 1132: 104-113.
9. Fidziańska A, Ryniewicz B, Shen XM, Engel AG. IBM-type inclusions in a patient with slow-channel syndrome caused by mutation in the AChR epsilon subunit. Neuromuscul Disord 2005; 15: 753-759.
10. Gomez CM, Maselli RA, Groshong J, Zayas R, Wollmann RL, Cens T, Charnet P. Active calcium accumulation underlies severe weakness in a panel of mice with slow channel syndrome. J Neurosci 2002; 22: 6447-6457.
11. Hughes BW, Kusner LL, Kamiński HJ. Molecular architecture of the neuromuscular junction. Muscle Nerve 2006; 33: 445-461.
12. Okada K, Inoue A, Okada M, Murata Y, Kakuta S, Jigami T, Kubo S, Shiraishi H, Eguchi K, Motomura M, Akiyama T, Iwakura Y, Higuchi O, Yamanashi Y. The muscle protein DOK-7 is essential for neuromuscular synaptogenesis. Science 2006; 312: 1802-1805.
13. Selcen D, Milone M, Shen XM, Harper CM, Stans AA, Wieben ED, Engel AG. DOK-7 myasthenia: phenotypic and molecular genetic studies in 16 patients. Ann Neurol 2008; 64: 71-87.
14. Willmann R, Fufrer C. Neuromuscular synaptogenesis, clustering of acetylocholine receptors revisted. Cell Mol Life Sci 2002; 59: 1296-1316.