Mechanisms of Neuromuscular Junction Dysfunction in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the death of upper and lower motor neurons. Numerous studies show that structural and functional impairments of neuromuscular junctions (NMJ) occur as early as the presymptomatic stage of ALS. NMJ involvement is independent and one of the primary events in ALS pathogenesis. Aim: *to review the data on characteristics and mechanisms of NMJ dysfunction at pre- and postsynaptic levels in ALS patients and a transgenic animal model of the disease. Furthermore, we report on the dysfunction of perisynaptic Schwann cells and impaired mechanisms of motor neuron and skeletal muscle interaction in ALS, with a focus on reviewed publications on targeting of molecular mechanisms underlying NMJ dysfunction and disruption in ALS. The NMJ may be a potential target for novel therapeutic approaches for ALS.*

Keywords: *amyotrophic lateral sclerosis; neuromuscular junction; nerve terminal; skeletal muscle; motor unit*

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Механизмы развития дисфункции нервно-мышечных синапсов при боковом амиотрофическом склерозе

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Аннотация

Боковой амиотрофический склероз (БАС) является прогрессирующим нейродегенеративным заболеванием, характеризующимся гибелью верхних и нижних мотонейронов. В многочисленных исследованиях показано, что структурно-функциональные нарушения нервно-мышечных синапсов (НМС) при БАС развиваются уже на досимптомной стадии болезни. Поражение НМС является самостоятельным и одним из первичных патогенетических процессов при БАС. Цель *обзора – анализ научных данных о характере поражения и механизмах нарушения функционирования НМС на пре- и постсинаптическом уровне при БАС у пациентов и в модели данного заболевания на трансгенных животных. Кроме того, представлены сведения о дисфункции перисинаптических шванновских клеток и о нарушении механизмов взаимодействия мотонейрона и скелетной мышцы при БАС. Особое внимание уделено анализу научных работ, связанных с коррекцией молекулярных механизмов, лежащих в основе дисфункции и деструкции НМС при БАС. Сделан вывод о том, что НМС может выступать потенциальной мишенью для разработки новых терапевтических подходов при БАС.*

Ключевые слова: *боковой амиотрофический склероз; нервно-мышечный синапс; нервное окончание; скелетная мышца; двигательная единица*

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disorder characterized by the death of upper and lower motor neurons [1]. ALS mostly begins with focal muscle weakness and hypotrophy that spread to adjacent myotomes along the cerebrospinal axis. Approximately one third of ALS patients start with the onset of bulbar symptoms, while two-thirds of patients have limb-onset disease [2, 3]. As ALS progresses, it leads to atrophy and paralysis of skeletal muscles, including the diaphragm. Average survival rate from the first diagnosis is 3 years [4]. Classic ALS is characterized by simultaneous upper and lower motor neuron involvement at one or more levels of the cerebrospinal axis, whereas atypical forms, such as primary lateral sclerosis, predominantly involve either upper or lower motor neurons [2, 5].

ALS is considered a multifactorial disease, with genetic, environmental, and age-dependent risk factors underlying its onset and development [6–9].

There are several hypotheses for neurodegeneration development and spread in ALS. The dying-forward hypothesis suggests that hyperexcitability of upper motor neurons is one of the initial events leading to glutamate excitotoxicity and lower motor neuron involvement [10–12]. Through the lens of the dying-back hypothesis the neuromuscular junction (NMJ), skeletal muscle, and distal axon play a crucial role in neurodegeneration initiation and development [9, 13, 14]. Alternatively, some investigators propose that upper and lower motor neuron degeneration proceeds independently [15, 16]. The involvement is thought to be independent and one of the primary events in ALS pathogenesis [9, 17, 18].

Aim: to review the data on characteristics and mechanisms of NMJ dysfunction at pre- and postsynaptic levels in ALS patients and a transgenic animal model of the disease.

NMJ Structure

The NMJ is a specialized synapse that connects the distal axon of a motor neuron with a skeletal muscle fiber. Perisynaptic Schwann cells (PSCs) cap the NMJ and regulate its structure and function. All the 3 elements of the NMJ (nerve terminal, postsynaptic membrane, and Schwann cell) are thought to be involved in ALS pathogenesis [9, 11].

A motor neuron and its innervated muscle fibers form a functional unit known as a motor unit (MU). Based on their contraction velocity and fatigability, MUs are categorized into slow (S), fast resistant to fatigue (FR), and fast fatigable (FF) [19, 20]. FR and FF fibers are innervated by fast motor neurons, whereas S fibers, by slow ones. Fast motor neurons have a larger soma size and axon diameter, a more branched dendritic tree, lower excitability, a higher rate of action potential generation, and faster axon conduction [21–24]. Experiments using mouse models of ALS revealed that FF MU involvement can be detected as early as the presymptomatic stage [25]; signs of FR MU involvement can be observed at symptom onset, while S MUs are affected at the late stage of the disease [26].

Involvement of NMJs and MUs in Patients

There is considerable evidence that the NMJ is affected at early stages of the disease in both ALS patients and multiple ALS models. Examinations of muscle biopsy specimens from ALS patients revealed pronounced fragmentation of end plates and their denervation [27]. Electron microscopy in ALS patients demonstrated a decrease in pre- and postsynaptic areas as well as in the percentage of nerve terminal mitochondria [28]. Expression of acetylcholine receptor subunits within the postsynaptic membrane is also reduced in ALS patients [29]. The study of muscle biopsy specimens revealed an increased proportion of slow muscle fibers, indicating selective vulnerability of fast MUs [30]. Electrophysiological studies also confirm that fast MUs are predominantly affected [31], except for extraocular muscles, which are spared in ALS [32].

In ALS patients, NMJ denervation and axon retraction may precede motor neuron degeneration and occur while spinal motor neurons and ventral roots remain intact [33]. Electrophysiological techniques confirm the NMJ involvement in ALS patients: amplitudes of miniature end-plate potentials and quantal content of end-plate potentials were shown to be decreased in muscle biopsy specimens of ALS patients at the early stages of the disease [34].

Transgenic Animal Models of ALS

Animal models significantly expanded possibilities of studying pathogenesis mechanisms and developing ALS therapies. Transgenic mouse lines expressing ALS-associated mutant human genes are usually used as model animals. Thus, a number of transgenic mouse models with ALS-linked gene mutations were developed to study the disease: *SOD1*, *FUS*, *C9orf72*, and *TARDBP* [9, 35]. These ALS models replicate clinical features and key pathogenesis mechanisms quite well, serving as an effective tool for studying the disease.

A mutation in the *SOD1* gene encoding superoxide dismutase 1 was the first identified genetic cause of ALS [36]. The first transgenic mouse model of ALS was a line of mice expressing the human *SOD1* with a *G93A* mutation [37]. This model is one of the most studied; it is actively used for preclinical studies and contributed to the introduction of riluzole and edaravone in ALS therapy [35]. The *SOD1*(*G93A*) model reproduces most mechanisms of ALS pathogenesis and demonstrates progressive motor neuron degeneration leading to paralysis and death in transgenic mice at 4–5 months of age[37].

Transgenic ALS model associated with the expression of a mutant *FUS* (fused in sarcoma) gene is widely used. *FUS* gene encodes a nuclear RNA/DNA-binding protein FUS [38]. The first transgenic models based on the FUS expression appeared in the early 2010s [39–41]. FUS transgenic mice reproduce such pathological processes in human ALS as accumulation of intracellular FUS aggregates, progressive death of motor neurons, skeletal muscle denervation with the development of paralysis and atrophy [42].

There is a transgenic model of ALS expressing a mutant *TARDBP* gene that encodes the DNA/RNA-binding protein TDP-43 [43]. Postmortem tissue changes in ALS patients include affected neurons and glia of the brain and spinal cord, characterized by the loss of nuclear TDP-43 and cytoplasmic accumulation of insoluble phosphorylated TDP-43[8]. Several TDP-43 models of ALS have been developed, and different phenotypes have been obtained [9, 44].

A *C9ORF72*-based genetic model of ALS was created. This gene encodes a protein found in neurons and other cells and involved in signaling in the nervous system [9]. Mouse models expressing the human *C9ORF72* repeats exhibit various pathological, functional, and behavioral characteristics of ALS [45].

Presynaptic NMJ Disorders

SOD1 Model

In SOD1 mice, NMJ involvement can be observed as early as the presymptomatic stage, preceding the first signs of ALS in motor neurons [46, 47]. Prior to obvious signs of NMJ denervation at the presymptomatic stage, one can observe altered nerve terminal morphology, as well as vacuolization and swelling of mitochondria with their decreased number in the presynaptic membrane [46, 47]. The changes primarily occur in the FF MUs [26, 46–48]. This model replicates several key mechanisms of ALS development, such as impaired axonal transport and mitochondrial dysfunction. Similar abnormalities and a decreased number of synaptic vesicles in the SOD1 model develop at the presymptomatic stage selectively in FF MUs, while FR and S MUs remain intact [26]. FR MU involvement becomes evident in the early symptomatic stage, while S MUs are affected in the late stage of the disease [26].

Impaired expression of synaptic proteins could be observed in this model. In SOD1 mice at the presymptomatic stage, we detected a significant decrease in the expression of presynaptic proteins, such as SNAP-25 and synapsin-1; after symptom onset we additionally observed a significant decrease in the synaptophysin expression [49]. Among the studied presynaptic proteins, SNAP-25 showed the most pronounced change: its expression reduced by ~50% compared with wild-type mice [49]. This vulnerability could be caused by SNAP-25 sensitivity to oxidative stress [50]. Oxidative stress in the presynaptic membrane also develops at the presymptomatic stage due to the decreased number of mitochondria and aberrant mitochondrial morphology [51]. Because of impaired axonal transport, the motor neuron cannot compensate for mitochondrial dysfunction in the nerve terminal [26].

The same ALS model was found to have impaired neuromuscular synaptic transmission. SOD1 mice have decreased quantal content of end-plate potentials and prolonged synaptic vesicle recycling both before and after symptoms onset [52]. Amplitude and frequency of miniature end-plate potentials was observed to be altered, with impaired synaptic transmission first becoming evident in FF MUs [25]. In this model, synaptic vesicle docking to the presynaptic membrane is also impaired [46, 47], which may result from impaired SNARE complex formation due to decreased SNAP-25 expression [49].

FUS Model

In a transgenic model overexpressing the human *FUS* (*hFUS*), NMJ denervation is accompanied by the preserved number of spinal motor neurons at the presymptomatic stage [53]. FUS aggregates are observed to accumulate in the presynaptic membrane of the NMJ [53]. Ultrastructural analysis in FUS mice revealed a decrease in the number of synaptic vesicles and nerve terminal mitochondria and their morphological abnormalities at the NMJ, while the postsynaptic membrane remains relatively intact [53]. However, another model, $FUS^{\Delta NLS/+}$, displayed reduced postsynaptic membrane area [54]. The selective vulnerability of fast MUs is also characteristic of FUS mice [55].

The FUS model is observed to have impaired expression of presynaptic proteins. Thus, we detected increased expression of synaptic proteins SNAP-25 and synapsin-1 in transgenic FUS(1-359) mice at the presymptomatic stage [17], whereas at the symptomatic stage a significant decrease in the expression of SNAP-25, synapsin-1, and synaptophysin was observed. The enhanced expression of some presynaptic proteins at the presymptomatic stage may be caused by messenger RNA stabilization due to FUS accumulation in the presynaptic membrane, which may affect local protein translation processes in the synapse [56, 57].

In the FUS model, impaired neuromuscular transmission is observed as early as the presymptomatic stage. In the FUS(1-359) model at the presymptomatic stage, we found a decrease in the amplitude of miniature (spontaneous) and evoked end-plate potentials, as well as in the rise time and half-decay time of miniature end-plate potentials compared with wild-type mice. Furthermore, there was a more significant decrease in the amplitude of end-plate potentials during high-frequency activity

(20 Hz) and a slower recovery of this amplitude after the stimulation in FUS(1-359) mice compared with wild-type mice. The FUS(1-359) mice also showed a decrease in the intensity of synaptic vesicle endocytosis induced by high-frequency synaptic stimulation (20 Hz) compared with wild-type mice [17].

Another study of FUS mice revealed a decrease in the amplitude of evoked motor responses that precedes morphological changes in pre- and postsynaptic membranes of the NMJ and axons, followed by loss of motor neurons [55].

Not all transgenic TDP-43 mice reliably reproduce the neuromuscular phenotype with muscle weakness, amyotrophy, and NMJ denervation. However, the TDP-43^{Q331K} model shows signs of impaired synaptic transmission at the presymptomatic stage (increased amplitude and decreased frequency of miniature end-plate potentials), as well as signs of NMJ polyinnervation [58].

Postsynaptic NMJ Disorders

Specific changes are observed in the postsynaptic membrane in ALS models. Transgenic SOD1 mice demonstrated morphologic changes: shortening of the end-plate folds [47]. In the SOD1 model, the expression of crucial postsynaptic structural proteins, such as nestin, dystrophin, LRP4, and rapsin, which are responsible for end-plate morphology and acetylcholine receptor clustering, is impaired at the symptomatic stage [59].

FUS mice have reduced postsynaptic membrane area, and these changes can be detected both at the presymptomatic stage [54] and only at the symptomatic stage [17]. Such changes are likely due to a direct effect of FUS on the expression of acetylcholine receptor subunits when FUS accumulates in the subsynaptic nuclei of skeletal muscle fibers [54].

Previously, the changes in skeletal muscles were thought to be secondary and solely the result of motor neuron degeneration. However, a number of studies suggest otherwise. Thus, in case of *SOD1* mutations, the accumulation of mutant superoxide dismutase 1 aggregates is observed in skeletal muscle at the presymptomatic stage [60] and leads to mitochondrial damage, resulting in impaired morphology and reduced number of mitochondria in the postsynaptic membrane at the presymptomatic stage, and oxidative stress [51]. The independent role of skeletal muscle in the ALS pathogenesis is also supported by the fact that, despite the prevention of spinal motor neuron death and their preserved number owing to p38 MAPK inhibitor, skeletal muscle denervation and atrophy still develop [46, 61].

Skeletal muscle can also act as a direct aggressor within the ALS pathogenesis. Selective overexpression of mutant *SOD1* results in NMJ involvement, distal axonopathy, and likely corticospinal tract damage, as evidenced by hyperreflexia and spasticity [62]. The impact of skeletal muscle may be mediated by the secretion of extracellular vesicles, which may exert neurotoxic effects by negatively affecting motor neuron survival and inhibiting axon growth [63, 64].

Skeletal muscle may also contribute to NMJ denervation by secreting Nogo-A factor, which is a chemorepellent, more properly a substance that repels the axon growth cone. This prevents effective reinnervation of the NMJ and contributes to progressive denervation of skeletal muscle [65]. The expression of this factor is elevated in ALS patients, with the level of expression correlating with the rate of disease progression [66]. Meanwhile, antibodies against Nogo-A notably delay disease progression in an ALS model [67].

Skeletal muscle metabolism is elevated in mSOD1 mice, leading to chronic energy deficits observed prior to amyotrophy and muscle denervation. Energy deficit and muscle hypermetabolism can lead to NMJ disruption, skeletal muscle denervation, and motor neuron death [68]. Dietary modification (a fat-enriched high-energy diet) extended life expectancy and motor neuron survival in a mouse model of ALS [69].

Involvement of PSCs in ALS

Apart from changes in the pre- and postsynaptic compartments of the NMJ, ALS patients also show pathological changes in the terminal PSCs [66]. The morphology of these cells is altered; outgrowth and intrusion in the synaptic cleft significantly reduce the available surface area of the postsynaptic membrane for neuromuscular transmission.

The SOD1(*G37R*) model showed that PSCs cannot produce an adequate response to NMJ degeneration (adoption of a phagocytic phenotype), nor can they guide nerve terminal sprouts. This impairs compensatory reinnervation and contributes to progressive denervation [70].

The SOD1(*G93A*) model revealed selective loss of PSCs and their macrophage infiltration in fast MUs at the presymptomatic stage [25, 71]. This observation also correlated with a reduced capacity of motor neurons innervating fast muscle fibers to reinnervate. The PSC involvement was also noted in the TDP43 model of ALS [72].

Moreover, the SOD1(*G93A*) model of ALS showed that PSCs in FF MUs are capable of *de novo* expression and secretion of the chemorepellent semaphorin 3A (Sema3A), which, like Nogo-A, repels the axon growth cone and leads to denervation, thereby contributing to the selective vulnerability of FF MUs [73]. R. Maimon et al. found that elevated Sema3A levels correlate with muscle denervation, with inhibition of Sema3A expression reducing the severity of NMJ and axon degeneration [74].

The role of PSCs in the ALS pathogenesis is also indirectly evidenced by the fact that masitinib administration in transgenic mice prevented loss of PSCs and delayed the disease [71]. At the same time, masitinib in combination with riluzole showed significant efficacy in ALS patients [75].

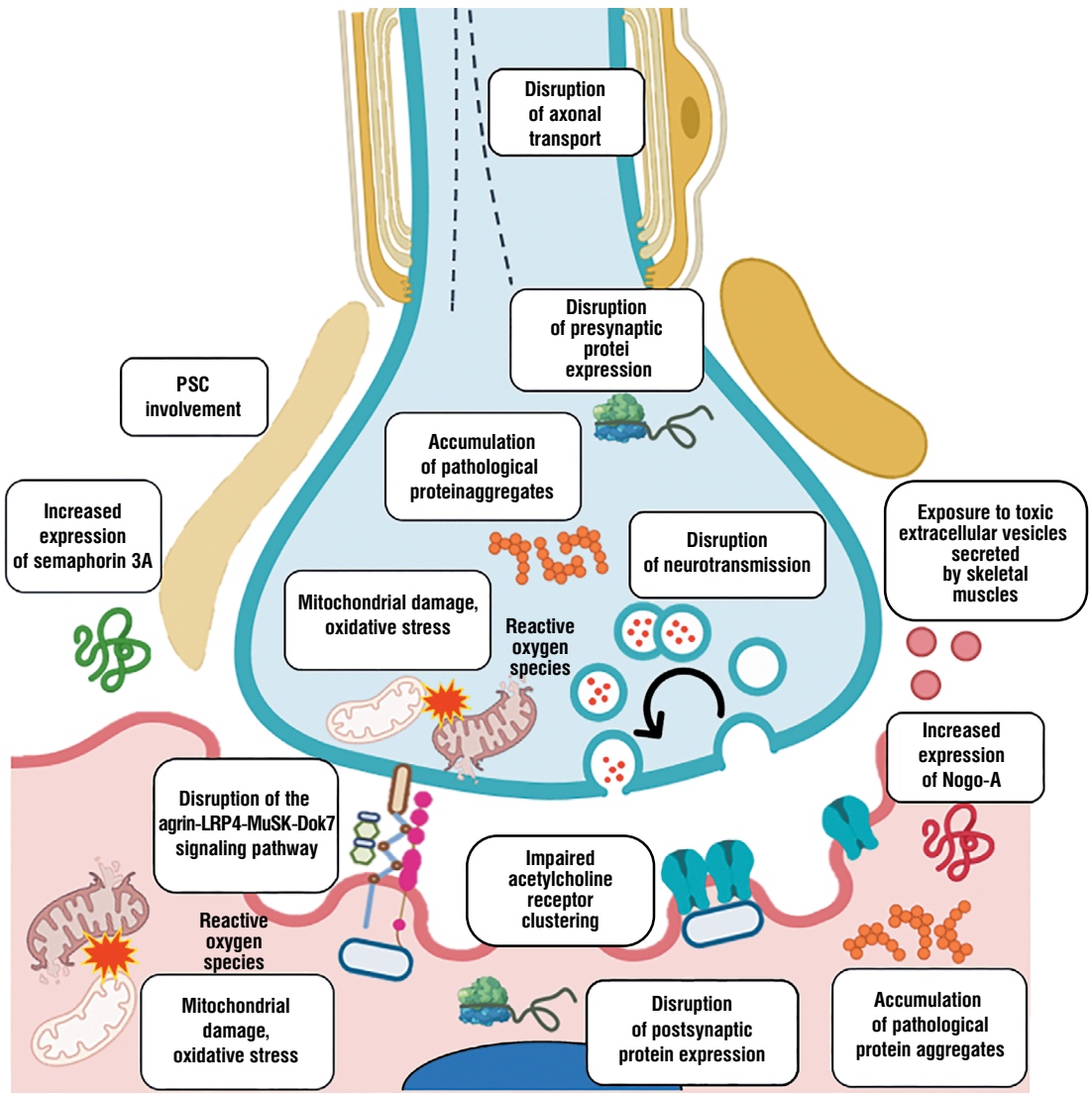
Impaired Mechanisms of Interaction Between Motor Neurons and Skeletal Muscles

In ALS, there are specific changes in each part of the NMJ: pre- and postsynaptic compartments, as well as the surrounding PSCs. Such changes inevitably lead to disruption of the motor neuron–skeletal muscle interaction, which in turn contributes

to further disease progression. Normally, when a motor neuron and a muscle fiber form a functional synapse, the formed MU begins to secrete a number of trophic and growth factors that ensure motor neuron survival, axon growth and regeneration, structural and functional stability of NMJs, differentiation and contractile properties of muscle fibers [76]. Such a secretome contains high concentrations of vascular endothelial growth factor, glial neurotrophic factor, brain-derived neurotrophic factor, neurotrophins-3 and -4, insulin-like growth factor-1, and insulin-like growth factor-3 binding protein. Innervated skeletal muscle was found to actively express the muscle-specific microRNA miR-206 [77]. miR-206 is thought to play a protective role by ensuring the survival of synaptic contacts and sprouting activity. High expression levels of miR-206 in ALS patients are associated with a slower rate of disease progression [78].

Disruption of the agrin-LRP4-MuSK signaling pathway may play a key part in NMJ involvement. Motor nerve terminals secrete agrin and low-density lipoprotein receptor-related protein-4 (LRP4), whereas skeletal muscle synthesizes rapsin, muscle-specific tyrosine kinase (MuSK), and the adaptor protein Dok-7. The interplay of these factors maintains the normal structure and functioning of the NMJ [79]. The signaling pathway regulates the acetylcholine receptor clustering on the postsynaptic membrane of the NMJ through a complex interaction of 3 proteins [80].

Internal processes in skeletal muscles may lead to disruption of the agrin-LRP4-MuSK signaling pathway. Thus, muscle fibers derived from induced pluripotent cells of ALS patients do not form functional NMJs with axons of healthy motor neurons, and there is no acetylcholine receptor clustering on the postsynaptic membrane in response to secreted agrin [30]. Disruption of MU functioning and integrity in such a case will inevitably lead to a deficiency of neurotrophic and growth factors, which will only contribute to



Pathogenic mechanisms of NMJ dysfunction in ALS and an ALS model.
The image was created with BioRender.com.

further disease progression [76]. The C9orf72 model of ALS demonstrated that poly(GA)-peptides formed as a result of the mutation inhibit the agrin-LRP4-MuSK signaling pathway, which leads to impaired neuromuscular transmission and damage to the pre- and postsynaptic membrane of the NMJs [81]. The SOD1(G93A) model revealed impaired MuSK transport into the postsynaptic membrane, resulting in NMJ involvement [82].

Activation and normalization of the agrin-LRP4-MuSK signaling pathway may have a positive effect on the ALS course. For instance, agrin overexpression in the TDP-43 model can prevent motor neuron death and preserve NMJs [83]. MuSK activation in this signaling pathway also has a beneficial effect by delaying denervation, promoting motor neuron survival, and increasing the lifespan of SOD1(G93A) transgenic mice [84–86]. Dok7 activation in the signaling pathway is also beneficial in terms of reducing the severity of NMJ degeneration and muscle atrophy, prolonging lifespan, and improving motor skills in the SOD1(G93A) transgenic model [87].

Conclusion

NMJ damage is an independent and early event in the ALS pathogenesis, as evidenced by the data from the studies in both transgenic animal models and ALS patients (Fig.). We should note that as early as the presymptomatic stage, a number of functional and structural disorders of the NMJ are observed in ALS models. All models with NMJ denervation demonstrated selective vulnerability of FF MUs in the early stages of the disease. In many models, the presynaptic compartment has been shown to be more vulnerable than the postsynaptic compartment. The identified functional disorders of the NMJ in ALS (according to the transgenic animal models data) indicate a decrease in the reliability of neuromuscular transmission both at low and high frequency. Structural abnormalities of NMJs in ALS include decreased area and fragmentation of synaptic contacts, altered expression of some synaptic proteins, etc.

Targeting molecular mechanisms underlying the dysfunction and destruction of NMJ in ALS garners a lot of interest. The NMJ may become a potential target for novel therapeutic approaches for ALS. We reviewed a number of quite successful attempts to modulate signaling pathways disrupted in the motor neuron–skeletal muscle system in ALS models [65–67, 73, 74, 77, 78].

Based on the findings obtained in transgenic animals, therapeutic methods aimed at increasing the agrin and miR-206 expression, activating MuSK, and suppressing the Sema3 and Nogo-A expression could potentially be quite effective in ALS. In addition to further study of the therapeutic potential of modulating the above-mentioned molecules, the possibility of their combination with drugs already in use (riluzole, edaravone) should be investigated to improve the efficacy of ALS therapy.

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