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REVIEWS

Review

Harmony Lost: Cell–Cell Communication at the Neuromuscular Junction in Motor Neuron Disease

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The neuromuscular junction (NMJ) is a specialized synapse that is the point of connection between motor neurons and skeletal muscle. Although developmental studies have established the importance of cell–cell communication at the NMJ for the integrity and full functionality of this synapse, the contribution of this structure as a primary driver in motor neuron disease pathogenesis remains uncertain. Here, we consider the biology of the NMJ and review emerging lines of investigation that are highlighting the importance of cell–cell interaction at the NMJ in spinal muscular atrophy (SMA), X-linked spinal and bulbar muscular atrophy (SBMA), and amyotrophic lateral sclerosis (ALS). Ongoing research may reveal NMJ targets and pathways whose therapeutic modulation will help slow the progression of motor neuron disease, offering a novel treatment paradigm for ALS, SBMA, SMA, and related disorders.

A Specialized Synapse for Nerve–Muscle Communication

The NMJ is a critical site, where electrical signals from the central nervous system are converted into physical movement, allowing an individual to navigate and interact with one's environment. More specifically, it is a unique and highly specialized synapse between lower motor neurons and skeletal muscle: an action potential arriving at the motor neuron axon terminal causes the presynaptic release of the neurotransmitter acetylcholine, which rapidly binds to postsynaptic nicotinic acetylcholine receptors on the muscle fiber surface, generating another action potential that triggers release of calcium from the sarcoplasmic reticulum, resulting in muscle contraction. Motor neurons and skeletal muscle are indispensable for life, and pathogenic conditions that affect one or both of these cell types can profoundly diminish quality of life, and if sufficiently severe, will cause death. Thus, research into the molecular mechanisms involved in the development, maintenance, and degeneration of the NMJ is essential for exploring therapeutic avenues in order to treat neuromuscular diseases.

An intriguing hypothesis has been put forward to explain how lower motor neurons degenerate in a number of important neuromuscular disorders. This so-called dying back hypothesis states that molecular disease pathology in neuromuscular disorders begins at the NMJ (Figure 1), and once the synapse is lost, a motor neuron axon will retract and degenerate in a process termed axonopathy [1], until its cell body (soma) dies. One observation in strong support of this hypothesis is that neuromuscular synaptic defects often precede motor neuron loss and clinical disease manifestations [2–5]. In this review, we first provide an overview of the current state of knowledge of basic NMJ biology in order to lay a foundation for understanding NMJ-relevant pathways and then examine the role of NMJ pathology in motor neuron disease, and discuss evidence for the dying back hypothesis in the pathogenesis of three neuromuscular disorders.

Highlights

The human motor neuron appears uniquely susceptible to distal perturbation at the NMJ. Thus, in diseases in which motor neurons are lost, their demise may begin at the nerve terminal, a phenomenon referred to as dying back.

Molecular mechanisms underpinning muscle–nerve uncoupling are only beginning to be understood but are critical to elucidate in order to obtain a better understanding of sporadic ALS, a devastating disease of unknown etiology.

Peripheral interventions aimed at stabilizing the NMJ could be broadly applicable to neuromuscular disease, and synergistic with targeted, disease-specific therapies, such as SMN protein-restoring drugs for SMA.

In SBMA, expression of the mutant androgen receptor protein in skeletal muscle has been established as the primary driver of lower motor neuron demise, revealing skeletal muscle as the obvious and highly accessible cellular target for future therapeutic intervention.

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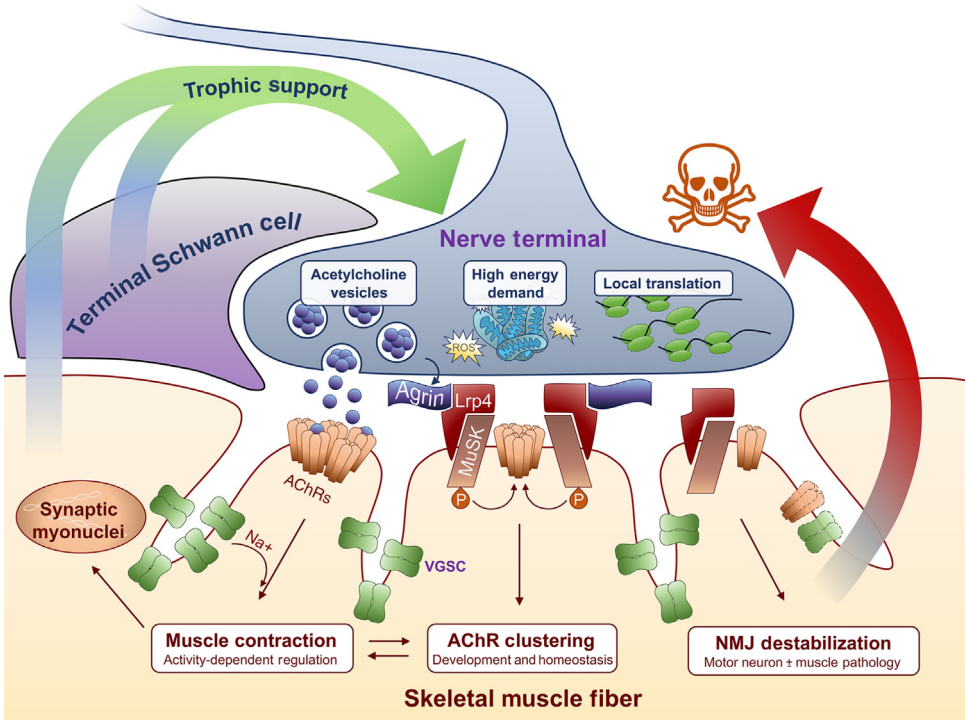


Figure 1. The Vertebrate NMJ. The NMJ transmits action potentials from lower motor neurons to skeletal muscle through secretion of the neurotransmitter acetylcholine, which binds to acetylcholine receptors, and initiates muscle depolarization by influx of sodium ions through VGSCs, culminating in contraction. Synaptic activity reinforces the specialization of synaptic myonuclei (also termed subsynaptic, perisynaptic, or postsynaptic myonuclei) towards maintaining the NMJ through transcription of NMJ-relevant machinery (such as *CHRN* genes) [121]. Central to establishing and maintaining the NMJ is the agrin–LRP4–MuSK axis: neurons secrete agrin, which binds to Lrp4 on the muscle surface, yielding phosphorylation of MuSK, and activated MuSK then induces clustering of AChRs through other cytoplasmic adaptor proteins. Thus, AChR clustering through agrin–LRP4–MuSK signaling is required for nerve-induced muscle contraction, and continued expression of postsynaptic components involved in this signaling cascade likewise relies on activity-dependent specification of synaptic myonuclei. As neurons require high levels of ATP, the high bioenergetic demand of proper motor neuron function is met by mitochondrial oxidative phosphorylation, resulting in reactive oxygen species that must be efficiently scavenged. Due to the great distance between nerve terminal and soma, neurons rely on local translation for maintaining the terminal [122,123]. Perturbations in skeletal muscle or motor neurons that result in failure of these mechanisms, such as loss of neural agrin causing subsequent declustering of postsynaptic machinery, results in destabilization of the NMJ. Under homeostatic conditions, motor neurons receive trophic support from muscle and nonmyelinating terminal Schwann cells [124,125] (Box 1), but in pathological conditions, loss of NMJ function contributes to dying back of motor neurons. Abbreviations: AChR, acetylcholine receptor; MuSK, muscle-specific kinase; NMJ, neuromuscular junction; VGSC, voltage-gated sodium channel.

Basic Biology and Main Players of the NMJ

It is well established that skeletal muscle relies on electrical input from motor neurons for homeostatic health, as skeletal muscle that suffers a loss of nerve input and becomes denervated through disease or spinal cord injury rapidly becomes severely atrophied and no longer maintains the structural organization of its contractile apparatus, culminating in invasion by adipose cells and fibrotic tissue (see Box 1 for a brief discussion of terminal Schwann cells, another crucial component of the NMJ). Some skeletal muscle cells persist and retain their regenerative capacity when exposed to exogenous electrical stimulation, even after decades of denervation [6]. Despite skeletal muscle dependence on motor neuron innervation for normal function and homeostatic health, the role of skeletal muscle in promoting motor neuron homeostasis is not fully understood,

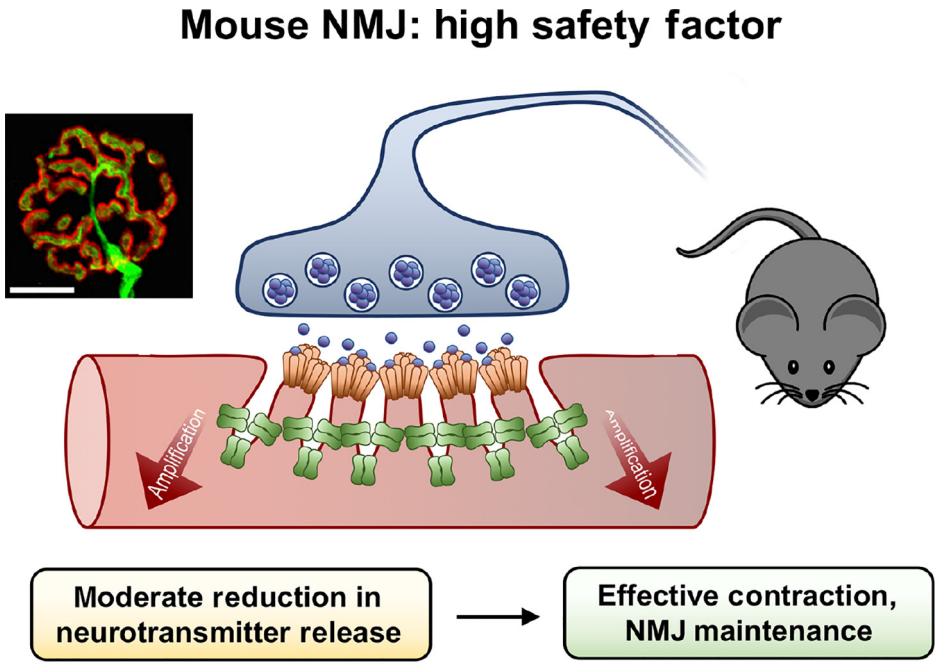
Box 1. The NMJ as a Tripartite Synapse

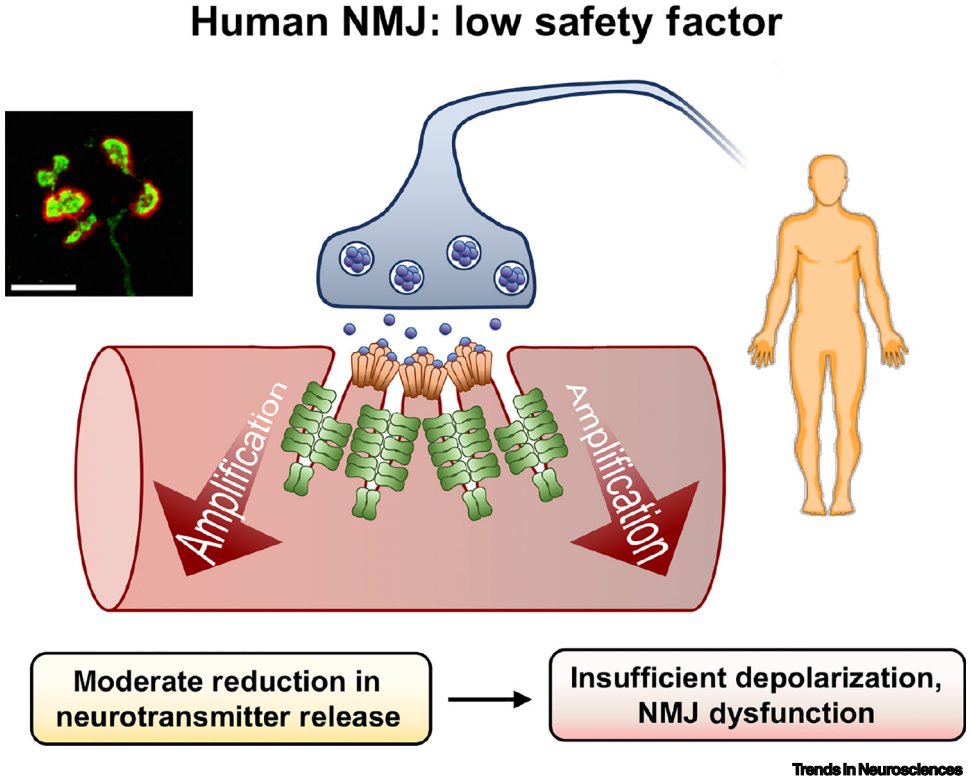
Yet another contributor to the health and function of the NMJ is the terminal Schwann cell (TSC, also called perisynaptic Schwann cell), a specialized, nonmyelinating glial cell that encapsulates the NMJ and appears indispensable for development, maintenance, and effective remodeling of the NMJ [113–115]) (see Figure 1 in main text). For example, tamoxifen-driven genetic ablation of Schwann cells in adult mice resulted in rapid and severe denervation leading to death [116]. However, as the Cre driver (*Plp1)* is broadly expressed in myelinating Schwann cells and brain oligodendrocytes [117], it is difficult to extrapolate the specific role of TSCs. An earlier, more specific TSC ablation study in frogs showed modest axon retraction and synaptic transmission defects at just 1 week postablation [118]. Furthermore, TSC abnormalities have been documented in certain cases of ALS, where cytosolic processes from Schwann cells extended into the synaptic cleft, potentially blocking neurotransmission [119], perhaps as an inappropriate outcome of attempting to guide reinnervating axons [120]. Although Schwann cells assuredly play a role in maintaining NMJ homeostasis, compared with their axon-myelinating cousins, little is known about their role in neuromuscular diseases and if they participate in the dying back of motor neurons. Hence, greater focus should be placed on understanding TSC biology in the context of NMJ dysfunction in the animal models discussed in this review (other than SOD1 mice), starting with the development of tools and methods to specifically target TSCs *in vivo*.

although their mutual dependence on one another is clear during mammalian development, where the survival of a subset of early motor neurons depends on skeletal muscle-derived trophic factors [7]. In agreement with this, genetic ablation of myofiber formation results in the eventual apoptosis of all motor neurons [8].

The formation and structure of the neuromuscular synapse are among the most extensively studied aspects of neurodevelopment, with some of the major players first discovered and purified from the electrical organs of rays and eels [9–11] and subsequent discernments of critical events using classical developmental biology models such as rodents, frog, chick, and fly (see [12,13] for a historical perspective). In mice, NMJ formation begins concurrently with primary myogenesis, when newly differentiated myofibers express primitive acetylcholine receptor (AChR) clusters that are denser along the middle region of the muscle through cell-autonomous mechanisms [14]. Such prepatterning primes muscle for the subsequent arrival of the developing motor neuron axon, which signals to skeletal muscle via secretion of agrin, a large proteoglycan. Agrin binds to its receptor LRP4 on the muscle fiber surface, allowing for agrin-dependent activation of muscle-specific kinase (MuSK), subsequent stabilization of AChR clusters solely at the synaptic domain such that extrasynaptic clusters dissipate, and eventual maturation into the characteristically elaborate pretzel-like shape of the NMJ [15]. This initial phase of NMJ formation results in polyinnervation, where each muscle fiber synapses with axons from multiple motor neurons, but extensive activity-dependent synaptic pruning then occurs postnatally until a muscle fiber receives input from only one motor neuron through a singular NMJ [16]. The canonical agrin–LRP4–MuSK axis is thought to also be required for homeostatic maintenance of the NMJ, as a subset of adult myasthenia gravis patients present with muscle weakness due to reduced AChR clustering, after developing autoantibodies to MuSK or less often to LRP4 (but not to the AChR itself) [17,18].

Detailed descriptions of NMJ biology can be found in several comprehensive reviews [13,19–22], but here we briefly highlight important differences between rodent and human NMJs (Figure 2). One key distinction is that despite the fact that human myofibers are nearly double the diameter of mouse myofibers, the human endplate has an area among the smallest of all vertebrates [23] and releases significantly less neurotransmitter per action potential (termed quantal content). In order to sufficiently depolarize adjacent skeletal muscle, greater infolding of the postsynaptic membrane in humans serves to amplify this neurotransmitter signal [24]. Consequently, human NMJs have been to proposed to have a smaller safety factor, meaning a modest decrease in quantal content is sufficient to cause dysfunction in neuromuscular transmission, whereas in mice, up to a 50% decrease in neurotransmitter release is well tolerated [23,25]. However,





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super-resolution microscopy of human and mouse NMJs labeled with an antibody against SNAP25 protein, a critical component of neurotransmitter vesicle exocytosis, revealed that despite their smaller size, human NMJs contain a denser concentration of SNAP25 proteins, such that overall neurotransmitter levels per NMJ are similar between humans and mice/rats [26]. While electrophysiological studies have largely agreed that rodent NMJs have a higher quantal content compared with human (~80 for mice/rats versus 20–50 for humans [27–31], this more recent work challenges the notion that human NMJs have a lower quantal content simply due to their smaller size. One possible explanation for this apparent discrepancy is that measurements in human tissue are scarce and thus not as well established as in rodents. However, as the SNAP25 investigation was largely based on snapshot imaging without functional experimentation [26], further work is needed to better understand human NMJ biology.

The mammalian NMJ is highly adaptive and dynamic; it is well documented that animal models in which synaptic transmission is partially blocked on the muscle surface (for example, through administration of AChR-binding neurotoxins at sublethal doses [27,32–34]) respond, at least initially, by increasing quantal content. Myasthenia gravis patients with antibodies against AChR show similar adaptive changes [29]. Thus, safety factor, being in part a function of presynaptic acetylcholine release, is somewhat flexible. Moreover, safety factor likely varies drastically between individuals and/or in the course of aging or diseases such as myasthenia gravis [35]. It is possible that individuals with a naturally low basal safety factor are more susceptible to perturbations at the NMJ, such as the age-related decline in proteostasis [36] as a broad example, and this lower adaptive threshold for catastrophic NMJ destabilization could partially explain the seemingly random nature of sporadic ALS. This idea, however, must be substantiated experimentally before a therapeutic intervention aimed at raising safety factor in vulnerable individuals can be considered. Assessment of the functional adaptiveness of the NMJ in humans is exceptionally difficult due to ethical concerns and tremendous variation in human biology, but the structural differences between rodents and humans are very clear, and such differences are important to consider when interpreting the results of neuromuscular studies performed in rodent models and extrapolating their relevance to human biology [37].

Dying Back of Motor Neurons in SMA is Cell-Autonomous

SMA is a group of neuromuscular diseases caused by death of lower motor neurons, resulting from loss of function of both autosomal copies of the *SMN1* gene [38] and, until recently, was the most common genetic cause of infant mortality in developed countries. The human genome contains identical duplications of *SMN* yielding a telomeric *SMN2* gene, but due to a point mutation in *SMN2*, the resulting transcript is often incorrectly spliced, prematurely terminated, and the resulting protein quickly degraded. Thus, only ~10% of functional survival motor neuron (SMN) protein is translated from the *SMN2* gene. The ability of *SMN2* to compensate for loss of *SMN1* through higher copy numbers in some SMA patients dictates the severity of SMA as follows: the most severe infantile-onset patients, called SMA type 1, typically have no more than two copies; intermediate type 2 SMA patients (onset at 6–18 months of age) usually possess

Figure 2. Comparison between Mouse and Human NMJs. Human NMJs are smaller than rodent NMJs; hence, less neurotransmitter is released per action potential (termed quantal content) compared with mouse NMJs. In order to trigger muscle depolarization from fewer ACh molecules, human NMJs contain deeper postsynaptic infolding, hosting more voltage-gated sodium channels that allow for a greater influx of sodium ions, thus serving to amplify the neurotransmitter action. Because human NMJs already have lower quantal content, any motor neuron pathology that further reduces ACh release has much more drastic consequence, namely synaptic transmission failure, while even a 50% reduction of quantal content has been reported to be tolerated by mouse NMJs and can still elicit muscle contraction [23,25]. Inset panels of mouse and human NMJs immunostained for presynaptic markers in green and Ach receptor in red are reproduced from [26]. Scale bars, 10 μm. Abbreviations: Ach, acetylcholine; NMJ, neuromuscular junction.

three copies of *SMN2*; and juvenile/adult-onset type 3 patients typically display ≥4 copies of *SMN2* [39]. Nusinersen, an antisense oligonucleotide therapy approved in late 2016, boosts the level of functional SMN protein produced from the *SMN2* gene by correcting its mis-splicing, and upon intrathecal injection, nusinersen can improve patient clinical outcomes for even the most severe forms of SMA. The success of nusinersen cannot be understated – infants with type I SMA, previously with zero chance of surviving their first year of life, are now well into their first decade of life as ostensibly healthy children [40]. Concerns over repeated intrathecal injections and continued lack of SMN protein in peripheral tissues encouraged the development of onasemnogene abeparvovec, an adeno-associated-virus-based gene replacement therapy that became the second FDA-approved treatment for SMA in 2019 [41]. Lastly, risdiplam, a small molecule splicing modifier aimed at increasing functional SMN protein made from *SMN2* both peripherally and in the CNS, was recently reported to improve motor function in SMA patients over 12 months of age [42]. In the face of these new therapies, the clinical landscape of SMA has been drastically altered (see [43,44] for recent reviews from a clinical perspective). Research and development on SMA are still by no means complete, as many questions about pathogenesis and progression remain, some of which we address later.

Despite the ubiquitous expression of SMN protein, pathogenesis of type I SMA appears to primarily stem from loss of SMN protein in motor neurons (Figure 3A). The selective vulnerability of neuronal subpopulations leading to the diverse manifestations of both rare and common neurodegenerative diseases is of interest to the field, and evidence for cell-autonomous motor neuron demise notwithstanding, SMA is no exception to this consideration. The normal functions of SMN protein are highly pleiotropic and include assembly of the spliceosome and other ribonucleoprotein complexes, trafficking and local translation of RNAs, endocytosis, and cytoskeleton dynamics, particularly at the growth cone [45–49]. Many of these cellular processes are critically important for motor neurons, which must maintain nerve terminals that are separated by up to a meter from their nuclei. NMJ pathology has been well studied in SMA and was among the earliest characterized phenotypes in mouse models [50], preceding loss of motor neuron soma in the spinal cord [2], and thus supporting the dying back hypothesis. Briefly, the dying back of motor neurons in severe type I SMA models seems to reflect the inability of SMN-deficient mice to properly mature the normal, embryonically formed NMJ in the postnatal period, presumably due to insufficient presynaptic activity and agrin release [51–56], although an alternative explanation may be required in the case of adult-onset SMA [57]. For more comprehensive reviews of NMJ pathology in SMA, we refer reader to [58–60]. *In vitro* experiments using human induced pluripotent stem cells (iPSCs) from SMA patients suggest a primary developmental defect, with reduced efficiency of motor neuron differentiation, neurite outgrowth, and ability to induce any AChR clustering when coculturing with C2C12 myotubes [61–63].

Several recent studies aimed to improve NMJ health in SMA mice through peripheral interventions, such as restoration of agrin levels. A transgenic approach in which the AChR-clustering (Z^+) agrin isoform is overexpressed in motor neurons in a mouse model of type I SMA results in a mild rescue of denervation and muscle size, but not motor neuron number or animal survival [64]. Considering SMN-deficient motor neurons have trafficking and local translation defects, it is unclear how much Z^+ agrin reached the NMJ, its functional target. However, another study injected an active agrin-derived peptide called NT-1654 into presymptomatic SMA pups and showed comparable mild rescue (also without increase in survival), but is more therapeutically relevant: NT-1654 is a pharmacological agent that can be administered systemically, and its delivery to wild-type mice do not elicit apparent negative outcomes [51]. A different approach sought to correct the defective presynaptic activity of SMA motor neurons by increasing calcium influx using systemically injected R-roscovitine, which slows inactivation of P/Q- and N-type

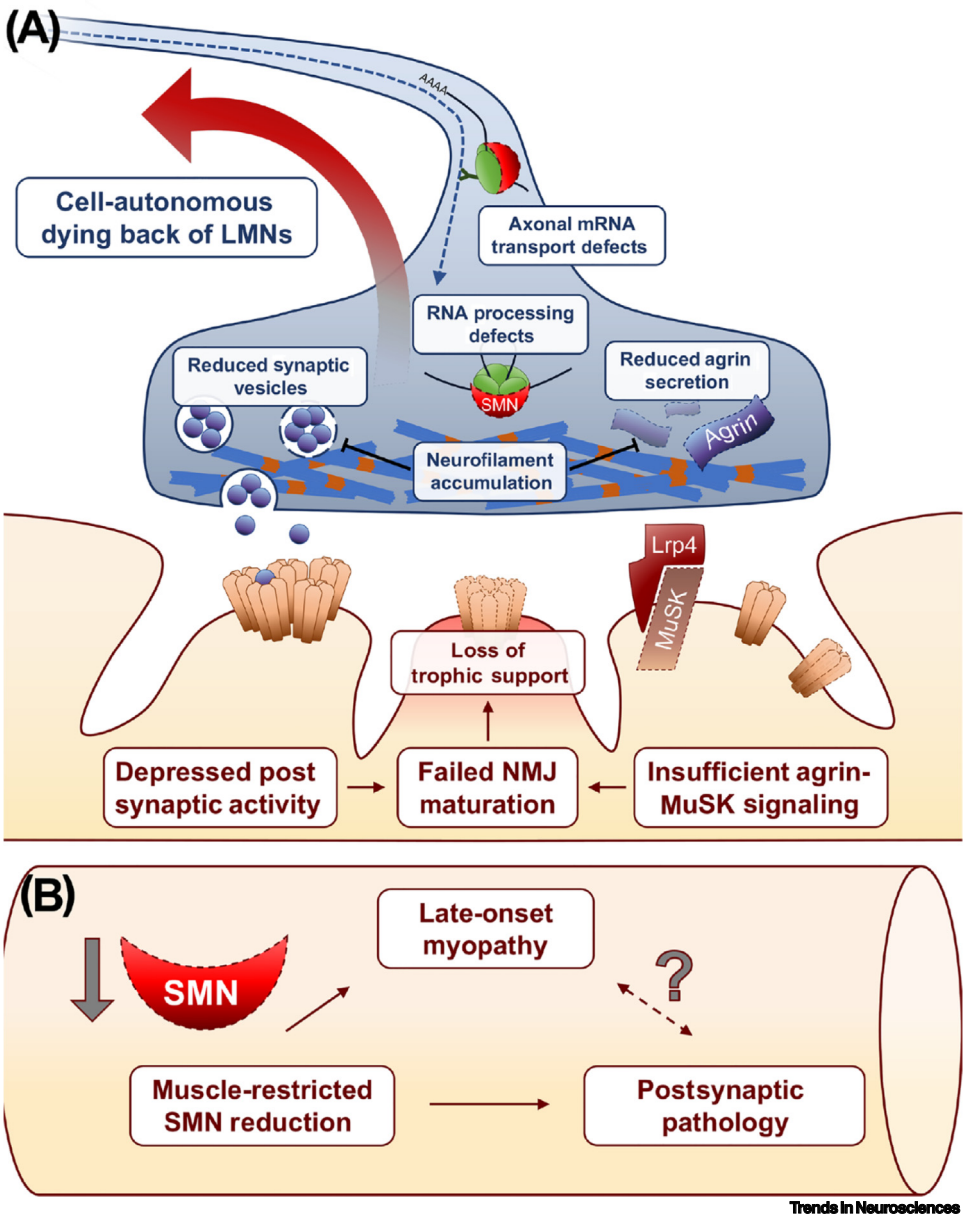

Figure 3. Mechanisms of NMJ Dysfunction in SMA. (A) SMN protein is involved in cellular processes that are critical to all cellular compartments. In the most severe type of SMA, infant mortality is caused by cell-autonomous dying back of LMNs that lack SMN. As SMN is heavily involved in axonal mRNA transport and RNA processing [45–49], loss of SMN is catastrophic to motor neurons and is concomitant with phenotypes that include fewer neurotransmitter vesicles and reduced agrin secretion that is exacerbated by accumulation of neurofilament at the nerve terminal. As a consequence, skeletal muscle lacks appropriate nerve input for NMJ maturation; the net result is depressed activity-dependent specialization of postsynaptic machinery (due to reduced acetylcholine) and insufficient acetylcholine receptor clustering (due to reduced agrin) [51–56]. As correction of these defects mildly improves severe SMA mouse phenotypes but does not prevent death, failure of postsynaptic maturation leading to loss of trophic support of the nerve terminal is likely a facilitator of dying back and not the driver. (B) SMN depletion restricted to the myogenic lineage can result in progressive late-onset myopathy and fragmented, dysfunctional NMJs [73]. The mechanisms behind these phenotypes and their interaction (dashed line) remain unknown (i.e., are they both directly caused by SMN loss, or is one simply a consequence of the other?). Consequently, cell-autonomous rescue of SMN-depleted motor neurons may uncover the involvement of other cell types and tissues in SMA disease processes and possibly pathogenesis. Abbreviations: LMN, lower motor neuron; MuSK, muscle-specific kinase; NMJ, neuromuscular junction; SMA, spinal muscular atrophy; SMN, survival motor neuron.

calcium channels. SMA pups that receive this small molecule *in utero* show improvements in neurotransmission at the NMJ and an increase in life span in parallel with a rescue of (or more likely, a delay in) motor neuron soma loss [65]. This is by no means an exhaustive list, and studies that take a variety of different approaches along these lines have also documented comparable improvements [64,66–71] (see [45] for a summary of clinical trials aimed at restoring muscle function in SMA).

Taken together, it is evident that drastic loss of motor neurons due to SMN deficiency cannot be circumvented solely by stabilization of NMJs via improvement of related downstream phenotypes. We recognize the general trend in the SMA field toward interventions in concert with antisense oligonucleotide (ASO)-induced restoration of SMN protein, with many groups using a low dose of ASO administered to severe models to reduce disease burden and model types II/III SMA. Due to the short therapeutic window of current treatments, additional disease-modifying avenues that could boost efficacy past that window would be beneficial, particularly for populations who may not have full access to newborn screening and healthcare (see Outstanding Questions). In other words, once a sublethal number of motor neurons have already died, can the remnants of the neuromuscular system be functionally improved to alleviate SMA symptoms? While not specifically attempting to prolong the therapeutic window of ASO therapy, the only identified study in mice that applied a peripheral intervention in concert with ASO treatment in type I SMA blocked the potent catabolic myokine myostatin, and showed dramatic rescue of muscle fiber size to levels nearly identical to those in wild-type mice, with substantial rescue of motor function, in contrast to ASO-only treated animals [72].

At present, we simply do not know how SMA patients receiving these life-saving treatments will fare in the long term, especially as their neuromuscular system will inevitably suffer age-related decline. The homeostatic role of SMN in peripheral tissues, including skeletal muscle, is likely to be of great importance, as intrathecal nusinersen has a negligible presence outside the CNS, and nonintegrating and nonreplicating onasemnogene abeparvovec, administered once to children under 2 years of age, would likely be eliminated from peripheral tissues undergoing rapid division and postnatal growth into adulthood. To highlight this concern, one group recently developed a mouse model in which only cells of the myogenic lineage were depleted of SMN and then documented late-onset myopathy in such mice, with highly fragmented and dysfunctional NMJs [73]. Thus, there may be a role for skeletal muscle in maintaining motor neuron health in SMA patients (Figure 3B), as the durability and sustainability of the currently approved effective treatments of SMA patients remain to be determined. To this end, development of splicing modifiers such as risdiplam is particularly important, provided such a therapeutic agent can be well tolerated throughout the life of the patient.

For Most Cases of ALS, the Basis of Dying Back Is Not Well Understood

ALS is a heterogeneous group of late-onset, rapidly progressive, and fatal neuromuscular diseases affecting upper and lower motor neurons. It exists on a broad clinical spectrum; at one end, patients present with distinctly lower motor neuron symptoms such as denervation, muscle atrophy, fasciculations, and cramping, while other patients with upper-motor-neuron-predominant symptoms exhibit spasticity, poor coordination, hypotonia, and hyperreflexia. Clinical categorization is complicated by site of onset (i.e., bulbar or spinal lower motor neurons) and concomitant presence of cognitive decline in up to 50% of cases [74], extending the spectrum into frontotemporal dementia (FTD) [75]. Unlike SMA, the etiology of ALS remains unknown for the majority of cases. Ninety to 95% of cases are categorized as sporadic ALS (SALS) due to a lack of a known genetic driver, and of the remaining 5–10% who are familial ALS (FALS), about 50% are caused by mutations in *C9ORF72*, *SOD1*, *TARDBP*, or *FUS*. However, such a

dichotomous classification should be done carefully, as certain known mutations do not display full penetrance. Furthermore, pathology in most ALS patients is likely initiated by a complex interaction between polygenic risk factors, environment, and lifestyle [76–78]. Due to a lack of faithful models because of the complex multifactorial nature and limitations of available postmortem human tissues, therapy development for ALS has been difficult, with only two FDA-approved therapies (edaravone and riluzole) that may modestly extend life expectancy, but not prevent fatality.

Since the discovery of mutations in *SOD1* in 1993 as the cause of ALS1, the primary model for the field has been the SOD1^{G93A} mouse, and many important findings have been made using this model. However, it is now apparent that the SOD1 mouse model has drawbacks and limitations: SOD1 mutations represent only 1–2% of all ALS cases; SOD1^{G93A} mice overexpress mutant SOD1 at supraphysiological levels; SOD1 mice do not fully recapitulate ALS-like upper motor neuron disease; and perhaps most importantly, SOD1 mice do not exhibit the most common pathogenic signature of ALS – nuclear depletion and cytoplasmic mislocalization of TAR DNA-binding protein 43 (TDP-43) in spinal cord motor neurons. Accordingly, continued over-reliance on this model may have stymied the development of effective therapies, and one should not extrapolate all SOD1^{G93A} mouse phenotypes to SALS or even to other forms of FALS. Fortunately, with the discovery of additional genes in FALS leading to novel animal models as well as the advent of iPSC technology for modeling ALS, the current and future state of ALS translational research is promising and exciting.

Similar to SMA, motor neuron demise via dying back, including the role of skeletal muscle, has been studied extensively in SOD1 mice and is reviewed elsewhere [79–81]. Numerous efforts to support the health of the NMJ have been pursued, including use of an antibody agonist to MuSK which prevented denervation at the NMJ in SOD1^{G93A} mice, but only extended lifespan by 7–10 days [82]. SOD1, as a free radical scavenger, is critically important for the health of most, if not all, cell types, especially those that have high bioenergetic demand such as neurons and muscle. Hence, similar to SMA, interventions that aim to stabilize the NMJ in SOD1 mice provide only modest delays in neuromuscular decline – just as SMN protein deficiency in SMA models cannot be rescued solely by peripheral interventions, this dramatic decompensation in various cell types in SOD1 mice likely cannot be rescued by such approaches either. Fortunately, an ASO against mutant *SOD1* is currently in development. Here, we instead focus on recent findings in other genetic ALS models.

Mice modeling more predominant forms of ALS have received increasing attention. The most common mutations leading to ALS/FTD are GGGGCC hexanucleotide repeat expansions in the first intron of the *C9ORF72* gene, and cause cellular toxicity via a toxic gain-of-function of non-ATG-translated dipeptides from the repeat track in combination with RNA gain-of-function and/or *C9ORF72* gene haploinsufficiency [83,84]. Several mouse models have been generated, but phenotypes are generally mild or produce widespread CNS pathology; hence, careful analysis of distal axonopathy has not been undertaken. However, a bacterial artificial chromosome (BAC) approach with ~500 repeats showed drastic denervation and loss of motor neurons [85], but as only end-stage data was reported, it is difficult to ascertain if NMJ loss preceded motor neuron death. Importantly, dipeptide repeat pathology is observed in the skeletal muscle of about half of studied C9-ALS patient samples [86] and in myofibers generated from C9-ALS patient iPSCs [87]. Another interesting parallel in ALS-associated gene phenotypes in skeletal muscle is seen with the RNA binding protein TDP-43. TDP-43 pathology, in which the predominantly nuclear protein is mislocalized into cytoplasmic granules, is perhaps the most unifying characteristic of postmortem ALS tissue: it has been observed in 97% of ALS cases [75], the

vast majority of which lack detectable mutations in the *TARDBP* gene. In human motor neurons, nuclear TDP-43 has been shown to be required for the correct splicing of stathmin-2 pre-mRNA, which encodes a microtubule stabilizer critical for axonal regeneration [88]; thus, its exclusion from the nucleus results in catastrophic axonal instability. TDP-43 in cytoplasmic granules has also been documented in myopathy [89,90] and was recently shown to be bound to mRNA transcripts for sarcomere components in the cytoplasm of regenerating muscle [89], which is in agreement with work in fly, mouse, and human neurons that showed TDP-43 is involved in transport of transcripts along the axon to distal compartments, including the NMJ [91]. TDP-43 also has a normal cytoplasmic function, but how that evolves into pathological aggregates in ALS (and FTD) remains unclear, and may involve the delicately balanced physical properties and dynamics of TDP-43-containing cytoplasmic structures that, if perturbed, prevent TDP-43 re-entry into the nucleus [92]. Regardless of the underlying mechanism, studies of TDP-43^{A315T} transgenic mice have documented a role for NMJ degeneration as a driver of disease progression, and have highlighted how compensatory presynaptic homeostatic plasticity can ameliorate motor neuron degeneration [93], underscoring the importance of the NMJ in ALS disease pathogenesis and progression.

More compelling evidence for a dying back of motor neurons has come from FUS ALS genetic models, in which mutations in the C-terminal nuclear localization portion of *FUS* are thought to lead to disruption of its RNA processing function, likely through a toxic gain-of-function [94]. A set of novel mouse models that express human mutant (R521C or P525L) *FUS* from the *MAPT* locus showed defects at the NMJ, including abnormal mitochondrial ultrastructure at both pre- and postsynaptic compartments and progressive denervation leading to motor dysfunction, which preceded any loss of motor neurons in the spinal cord by ~10 days [95]. However, considering these mice were reported to have a normal lifespan, a 10-day difference should be carefully interpreted. Another group that replaced endogenous mouse *Fus* with human normal or ALS-mutant *FUS* provided important mechanistic insights into the effect of mutant *FUS* on neurons by documenting drastic suppression of axonal translation in sciatic nerves, presumably through increased phosphorylation of eukaryotic initiation factor 2α, that was correlated with increased intra-axonal accumulation of mutant FUS protein [96]. Taken together with the observation that in nontransgenic mice, FUS localizes strongly to the NMJ [97], a role for FUS in NMJ biology is emerging. A distinct and equally intriguing mechanistic finding was described recently using yet another FUS-ALS model in which the entire nuclear localization signal (NLS) was eliminated, thereby producing a severe, perinatal lethal phenotype. These results provide important insights into the mechanistic basis of FUS-driven pathology, but their relevance to SALS remains unclear, even though certain FUS mouse models, such as heterozygous FUS$^{\Delta NLS/+}$ mice, develop adult-onset neuromuscular disease [98]. Contrary to the study reporting FUS protein localization to the NMJ [97], immunostaining of NMJs from adult-onset animals did not show localization to the endplate *per se*, instead showing FUS enrichment in myonuclei associated with the nerve terminal, termed subsynaptic synaptic nuclei [98] (also referred to as perisynaptic, postsynaptic, or simply synaptic myonuclei). Strong *in vitro* data in which C2C12 muscle cells were used to probe the molecular role of FUS established that normal (nuclear) FUS is critical for promoting nicotinic cholinergic receptor gene expression in muscle cells in concert with the transcription factor ERM (also known as Etv5), thus providing a mechanistic link between loss of nuclear FUS as is seen in ALS patients and the destabilization of the NMJ by way of reduced postsynaptic AChR machinery. We hope that such reports will prompt investigators to carefully dissect NMJ pathology and perform experiments aimed at stabilizing the NMJ in their models, as has been done extensively for SOD1 mice, to assess the contribution of NMJ dysfunction to ALS disease onset and progression.

Myopathy in SBMA Drives Non-Cell Autonomous Motor Neuron Degeneration

Like SMA, SBMA is a monogenic neuromuscular disorder, but instead is a sex-limited dominant gain-of-function disease, caused by repeat expansions of the CAG codon (translated into glutamine, [Q]) in the first exon of the androgen receptor (*AR*) gene on the X chromosome. Akin to ALS, SBMA is late onset and no effective therapy has been developed, but unlike either SMA or ALS, SBMA is slowly progressive and only sometimes fatal, although SBMA patients can suffer significant disability, as any perturbation to the neuromuscular system can affect quality of life. Indeed, many SBMA patients eventually require ambulatory assistance, and due to the involvement of bulbar muscles, difficulty in swallowing confers a higher risk for choking and aspiration pneumonia, which accounts for observed disease-dependent mortality [99]. Another unique feature of SBMA is that the role of skeletal muscle in disease pathogenesis is well established. This was a surprising development, because the primary clinical phenotype of SBMA has historically been lower motor neuron degeneration leading to progressive muscle weakness, but a series of studies over the past decade successfully challenged this neurocentric dogma. One critical finding has been that SBMA patient biopsies show obvious evidence of myopathy (i.e., central nucleation, fiber splitting and necrosis, and lobular fibers) that is distinct from neurogenic changes (i.e., denervation) in skeletal muscle, and laboratory testing of SBMA patients yields significantly increased serum creatine kinase (CK) levels [100]. Similar findings have been reported in a small subset of ALS patient biopsies, albeit to a lesser extent [101]. Furthermore, SBMA patients do not display appreciable levels of neurofilaments in their blood [102], which is a biomarker for neuronal damage used in ALS and other neurodegenerative diseases [103], and ASO-based silencing of mutant AR in only the periphery is sufficient to rescue disease phenotypes in SBMA mouse models [104].

Recent work by our group definitively implicated polyQ-AR expression in skeletal muscle as the cellular basis for SBMA motor neuron degeneration. Using BAC transgenic mice where expression of mutant human AR containing 121 CAG repeats can be excised in a tissue-specific manner by Cre recombinase (i.e., the BAC fxAR121 line), we recapitulated the defining pathological hallmarks of SBMA, including progressive muscle weakness and motor defects limited to males, in combination with myopathic changes and lower motor neuron degeneration [105]. However, when we bred BAC fxAR121 mice to mice expressing Cre recombinase driven by a skeletal muscle-specific promoter (HSA-Cre), the resulting double transgenic mice displayed a complete amelioration of SBMA symptoms and lived a normal lifespan, with preservation of motor neuron axon area, despite continued expression of mutant polyQ-AR protein in motor neurons [105]. These findings indicate that in SBMA, not only does motor neuron degeneration begin at the NMJ, but also polyQ-AR in skeletal muscle is required to confer a toxic gain-of-function (possibly in combination with a partial loss-of-function) that drives the resulting neuropathology (Figure 4). Whether the mechanism underlying this phenomenon, which is currently unknown, is unique to SBMA or can be harnessed as a therapeutic intervention to improve the health of the NMJ for other neuromuscular diseases such as ALS is of great interest and paramount importance for translational research in this field.

Concluding Remarks

A major risk factor for ALS, SBMA, and late-onset SMA is aging, and many cellular phenotypes highlighted in this review have been characterized in rodents during the cellular process of age-related muscle decline, which is termed sarcopenia [106]. For example, sarcopenic animals have extensively fragmented NMJs with mislocalized AChRs and reduced junctional folds, dysfunctional presynaptic input, and degenerated axons, culminating in denervation [107–109]. Loss of muscle mass appears to precede NMJ pathology and the loss of motor neuron soma is apparently absent [110], suggesting that unlike ALS, neuromuscular decline in sarcopenia is

Outstanding Questions

For infants born with type I SMA, can the therapeutic window of SMN-restoring therapies be extended by additional, NMJ-stabilizing interventions?

For adolescents/adults with milder SMA and type I patients that receive lifesaving therapy early, what are the long-term implications of treatment in the face of age-related NMJ decline?

What phenotypes will result from continued SMN loss-of-function in peripheral tissues and how can peripheral pathology, if it exists and impairs the nervous system, be mitigated?

How universal is dying back of motor neurons in ALS, an incredibly heterogeneous group of diseases?

Should ALS patients with primary NMJ dysfunction be identified and treated differently than ALS patients with predominantly upper motor neuron pathology? If so, how can we develop therapies tailored to the pathological predilections of different ALS patients?

How does mutant AR in skeletal muscle drive lower motor neuron dysfunction and degeneration in SBMA?

If this mechanism can be delineated and then therapeutically rescued, could the resulting treatment paradigm be extended to other neuromuscular disorders, such as ALS and SMA?

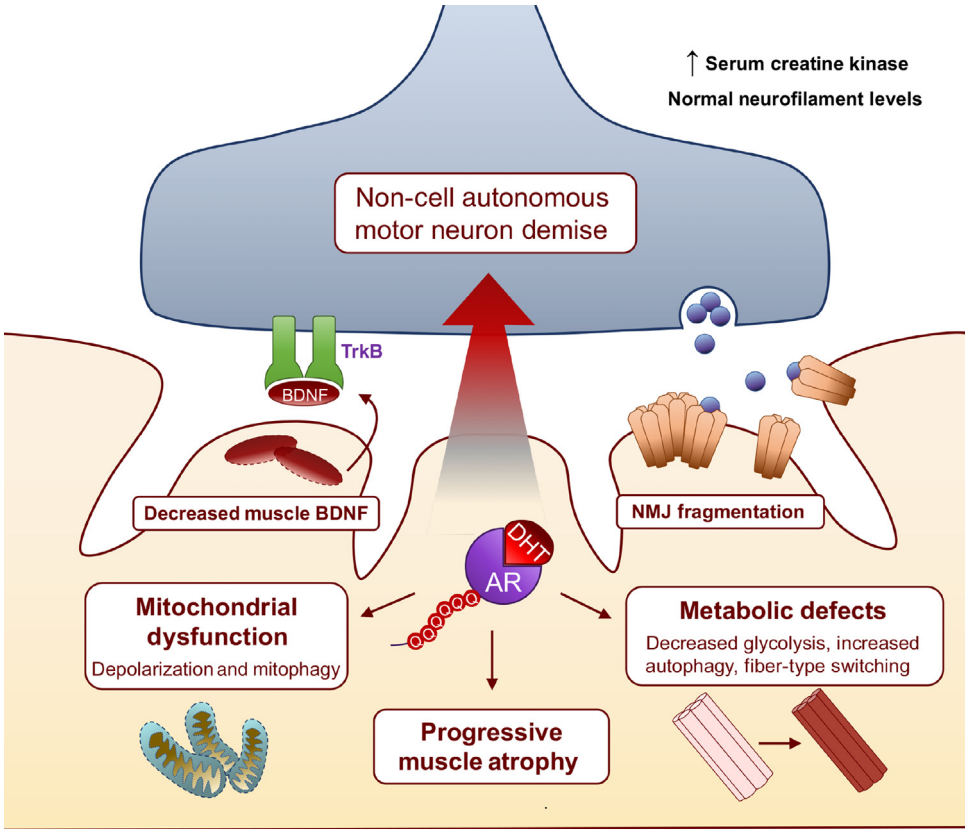

Figure 4. Myopathy in SBMA Drives Motor Neuron Demise. Polyglutamine-expanded AR, the genetic cause of SBMA, elicits cell-autonomous myopathy that is highlighted by elevated serum creatine kinase levels in SBMA patients [100], due to leakage of creatine kinase from damaged muscle fibers, without an increase in neurofilaments [102], which is released from dying neurons. The binding of mutant AR to its ligand, DHT, is required for pathology, as female carriers of the mutant gene are clinically normal due to their naturally low amount of circulating androgen [126]. Pathogenic phenotypes in SBMA muscle primarily center around bioenergetics and metabolism, including depolarized mitochondria (based upon reported direct physical interaction with mutant AR protein [127]) that undergo excessive mitophagy [128], decreased glycolysis associated with glycolytic-to-oxidative fiber type switching [129], and pathologically increased autophagy [129–131]. Furthermore, NMJs in SBMA muscle are fragmented [132], and BDNF to motor neurons [133]. Despite these findings, it is unknown how mutant AR in skeletal muscle leads to motor neuron degeneration in SBMA. Abbreviations: AR, androgen receptor; BDNF, brain-derived neurotrophic factor; DHT, dihydrotestosterone; NMJ, neuromuscular junction; SBMA, spinal and bulbar muscular atrophy.

driven by skeletal muscle. In humans, a role for NMJ dysfunction in sarcopenia is even less established. From a morphological standpoint, recent evidence suggests that the human NMJ is exceptionally stable with age [26]. However, the aforementioned report examined only 20 individuals across ages that span >70 years and none of the older donors were reported to be sarcopenic. Furthermore, one of the most well described features of rodent NMJs in aging is increased fragmentation, while the human NMJ is already naturally highly fragmented, so functional deficits, rather than morphological changes, are more likely to be evident, but are not mutually exclusive. Nevertheless, we applaud the authors for documenting these findings in human tissue, as such studies are exceptionally rare (but highly informative), so we hope the field is encouraged to carefully explore human NMJ biology, especially in the context of neurodegeneration. Additionally, it is well established that physical activity is highly protective against sarcopenia [111], yet even elite athletes succumb to SALS. Whether and how age-related

pathways driving sarcopenia intersect with the pathobiology of motor neuron disease remains unclear; hence, therapeutically intervening to stem the process of sarcopenia as an approach to treating motor neuron disease should be explored, but its utility remains uncertain.

In summary, early-stage pathology at the NMJ leading to a dying back of motor neuron axons has been well documented in various models of neuromuscular disease, and is increasingly recognized as a crucial step in the pathogenic cascade, although the molecular mechanisms underpinning this phenomenon are not understood.

A thorough understanding of the role of the NMJ as a principal arbiter of disease pathogenesis has been hampered by transgenic artifacts in animal models and inherent differences between rodent and human NMJs; however, advances in the development of iPSC-based models will enable the field to better examine the role of aberrant cell–cell communication at the NMJ. Despite numerous studies on motor neurons generated from SMA, SBMA, FALS, and even SALS patient iPSCs [112], recapitulating the human NMJ itself from iPSC derivatives remains challenging, due to the technically difficult nature of iPSC-derived skeletal muscle and motor neuron cocultures, and in particular, efficient and homogeneous differentiation of iPSCs into myotubes. Undoubtedly, advances in both 2D and 3D modeling of the NMJ in motor neuron – skeletal muscle cocultures will set the stage for the field to more definitively address underlying mechanisms of pathological cell–cell communication at the NMJ, and identify rational targets and pathways for therapeutic modulation.

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References

1. Yaron, A. and Schuldiner, O. (2016) Common and divergent mechanisms in developmental neuronal remodeling and dying back neurodegeneration. *Curr. Biol.* 26, R628–R639
2. Courtney, N.L. *et al.* (2019) Reduced P53 levels ameliorate neuromuscular junction loss without affecting motor neuron pathology in a mouse model of spinal muscular atrophy. *Cell Death Dis.* 10, 515
3. Fischer, L.R. *et al.* (2004) Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. *Exp. Neurol.* 185, 232–240
4. Frey, D. *et al.* (2000) Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. *J. Neurosci.* 20, 2534–2542
5. Martineau, E. *et al.* (2018) Dynamic neuromuscular remodeling precedes motor-unit loss in a mouse model of ALS. *Elife* 7, e41973
6. Kern, H. *et al.* (2004) Long-term denervation in humans causes degeneration of both contractile and excitation-contraction coupling apparatus, which is reversible by functional electrical stimulation (FES): a role for myofiber regeneration? *J. Neuropathol. Exp. Neurol.* 63, 919–931
7. Kanning, K.C. *et al.* (2010) Motor neuron diversity in development and disease. *Annu. Rev. Neurosci.* 33, 409–440
8. Kablar, B. and Rudnicki, M.A. (1999) Development in the absence of skeletal muscle results in the sequential ablation of motor neurons from the spinal cord to the brain. *Dev. Biol.* 208, 93–109
9. Jennings, C.G. *et al.* (1993) Muscle-specific trk-related receptor with a kringle domain defines a distinct class of receptor tyrosine kinases. *Proc. Natl. Acad. Sci. U. S. A.* 90, 2895–2899
10. Miledi, R. *et al.* (1971) Isolation of the cholinergic receptor protein of Torpedo electric tissue. *Nature* 229, 554–557
11. Godfrey, E.W. *et al.* (1984) Components of Torpedo electric organ and muscle that cause aggregation of acetylcholine receptors on cultured muscle cells. *J. Cell Biol.* 99, 615–627
12. Hall, Z.W. and Sanes, J.R. (1993) Synaptic structure and development: the neuromuscular junction. *Cell* 72, 99–121
13. Sanes, J.R. and Lichtman, J.W. (1999) Development of the vertebrate neuromuscular junction. *Annu. Rev. Neurosci.* 22, 389–442
14. Yang, X. *et al.* (2001) Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. *Neuron* 30, 399–410
15. Kim, N. *et al.* (2008) Lrp4 is a receptor for Agrin and forms a complex with MuSK. *Cell* 135, 334–342
16. Brown, M.C. *et al.* (1976) Polyneuronal innervation of skeletal muscle in new-born rats and its elimination during maturation. *J. Physiol.* 261, 387–422
17. Pevzner, A. *et al.* (2012) Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. *J. Neurol.* 259, 427–435
18. Herbst, R. (2020) MuSk function during health and disease. *Neurosci. Lett.* 716, 134676
19. Li, L. *et al.* (2018) Neuromuscular junction formation, aging, and disorders. *Annu. Rev. Physiol.* 80, 159–188
20. Tintignac, L.A. *et al.* (2015) Mechanisms regulating neuromuscular junction development and function and causes of muscle wasting. *Physiol. Rev.* 95, 809–852
21. Wu, H. *et al.* (2010) To build a synapse: signaling pathways in neuromuscular junction assembly. *Development* 137, 1017–1033
22. Shi, L. *et al.* (2012) Molecular mechanisms underlying maturation and maintenance of the vertebrate neuromuscular junction. *Trends Neurosci.* 35, 441–453
23. Slater, C.R. (2017) The structure of human neuromuscular junctions: some unanswered molecular questions. *Int. J. Mol. Sci.* 18

24. Martin, A.R. (1994) Amplification of neuromuscular transmission by postjunctional folds. *Proc. Biol. Sci.* 258, 321–326
25. Wood, S.J. and Slater, C.R. (2001) Safety factor at the neuromuscular junction. *Prog. Neurobiol.* 64, 393–429
26. Jones, R.A. *et al.* (2017) Cellular and molecular anatomy of the human neuromuscular junction. *Cell Rep.* 21, 2348–2356
27. Wang, X. *et al.* (2016) Reversible recruitment of a homeostatic reserve pool of synaptic vesicles underlies rapid homeostatic plasticity of quantal content. *J. Neurosci.* 36, 828–836
28. Slater, C.R. *et al.* (2006) Pre- and post-synaptic abnormalities associated with impaired neuromuscular transmission in a group of patients with 'limb-girdle myasthenia'. *Brain* 129, 2061–2076
29. Plomp, J.J. *et al.* (1995) Acetylcholine release in myasthenia gravis: regulation at single end-plate level. *Ann. Neurol.* 37, 627–636
30. Fong, S.W. *et al.* (2010) TGF-beta2 alters the characteristics of the neuromuscular junction by regulating presynaptic quantal size. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13515–13519
31. Maselli, R.A. *et al.* (1993) Neuromuscular transmission in amyotrophic lateral sclerosis. *Muscle Nerve* 16, 1193–1203
32. Plomp, J.J. *et al.* (1992) Adaptation of quantal content to decreased postsynaptic sensitivity at single endplates in alpha-bungarotoxin-treated rats. *J. Physiol.* 458, 487–499
33. Plomp, J.J. *et al.* (1994) The upregulation of acetylcholine release at endplates of alpha-bungarotoxin-treated rats: its dependency on calcium. *J. Physiol.* 478, 125–136
34. Wang, X. *et al.* (2010) Activity-dependent regulation of the binomial parameters p and n at the mouse neuromuscular junction *in vivo*. *J. Neurophysiol.* 104, 2352–2358
35. Ruff, R.L. and Lennon, V.A. (2008) How myasthenia gravis alters the safety factor for neuromuscular transmission. *J. Neuroimmunol.* 201-202, 13–20
36. Kaushik, S. and Cuervo, A.M. (2015) Proteostasis and aging. *Nat. Med.* 21, 1406–1415
37. Fisher, E.M.C. and Bannerman, D.M. (2019) Mouse models of neurodegeneration: know your question, know your mouse. *Sci. Transl. Med.* 11
38. Lefebvre, S. *et al.* (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80, 155–165
39. Calucho, M. *et al.* (2018) Correlation between SMA type and SMN2 copy number revisited: an analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscul. Disord.* 28, 208–215
40. Finkel, R.S. *et al.* (2017) Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N. Engl. J. Med.* 377, 1723–1732
41. Al-Zaidy, S. *et al.* (2019) Health outcomes in spinal muscular atrophy type 1 following AVXS-101 gene replacement therapy. *Pediatr. Pulmonol.* 54, 179–185
42. Mercuri, E. *et al.* (2019) Update from SUNFISH part 1: safety, tolerability and PK/PD from the dose-finding study, including exploratory efficacy data in patients with type 2 or 3 spinal muscular atrophy (SMA) treated with risdiplam (RG7916) (S25.007). *Neurology* 92 S25.007
43. Schorling, D.C. *et al.* (2020) Advances in treatment of spinal muscular atrophy - new phenotypes, new challenges, new implications for care. *J. Neuromuscul. Dis.* 7, 1–13
44. Dangouloff, T. and Servais, L. (2019) Clinical evidence supporting early treatment of patients with spinal muscular atrophy: current perspectives. *Ther. Clin. Risk Manag.* 15, 1153–1161
45. Wirth, B. *et al.* (2020) Twenty-five years of spinal muscular atrophy research: from phenotype to genotype to therapy, and what comes next. *Annu. Rev. Genomics Hum. Genet.* 21. https://doi.org/10.1146/annurev-genom-102319-103602 Published online January 31, 2020
46. Chaytow, H. *et al.* (2018) The role of survival motor neuron protein (SMN) in protein homeostasis. *Cell. Mol. Life Sci.* 75, 3877–3894
47. Fallini, C. *et al.* (2016) Deficiency of the survival of motor neuron protein impairs mrna localization and local translation in the growth cone of motor neurons. *J. Neurosci.* 36, 3811–3820
48. Singh, R.N. *et al.* (2017) Diverse role of survival motor neuron protein. *Biochim. Biophys. Acta Gene Regul. Mech.* 1860, 299–315
49. So, B.R.Zhang, Z.Dreyfuss, G. (2017) The function of survival motor neuron complex and its role in spinal muscular atrophy pathogenesis. In *Spinal Muscular Atrophy*, pp. 99–111, Academic Press
50. Cifuentes-Diaz, C. *et al.* (2002) Neurofilament accumulation at the motor endplate and lack of axonal sprouting in a spinal muscular atrophy mouse model. *Hum. Mol. Genet.* 11, 1439–1447
51. Boido, M. *et al.* (2018) Increasing agrin function antagonizes muscle atrophy and motor impairment in spinal muscular atrophy. *Front. Cell. Neurosci.* 12, 17
52. Kong, L. *et al.* (2009) Impaired synaptic vesicle release and immaturity of neuromuscular junctions in spinal muscular atrophy mice. *J. Neurosci.* 29, 842–851
53. Kariya, S. *et al.* (2008) Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy. *Hum. Mol. Genet.* 17, 2552–2569
54. Zhang, Z. *et al.* (2013) Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. *Proc. Natl. Acad. Sci. U. S. A.* 110, 19348–19353
55. Martinez, T.L. *et al.* (2012) Survival motor neuron protein in motor neurons determines synaptic integrity in spinal muscular atrophy. *J. Neurosci.* 32, 8703–8715
56. Gogliotti, R.G. *et al.* (2012) Motor neuron rescue in spinal muscular atrophy mice demonstrates that sensory-motor defects are a consequence, not a cause, of motor neuron dysfunction. *J. Neurosci.* 32, 3818–3829
57. Udina, E. *et al.* (2017) Compensatory axon sprouting for very slow axonal die-back in a transgenic model of spinal muscular atrophy type III. *J. Physiol.* 595, 1815–1829
58. Boido, M. and Vercelli, A. (2016) Neuromuscular junctions as key contributors and therapeutic targets in spinal muscular atrophy. *Front. Neuroanat.* 10, 6
59. Vrbova, G. and Slawinska, U. (2018) Critical period of neuromuscular development: importance for a new treatment of SMA. *Neuromuscul. Disord.* 28, 385–393
60. Park, G.H. *et al.* (2010) Spinal muscular atrophy: new and emerging insights from model mice. *Curr. Neurol. Neurosci. Rep.* 10, 108–117
61. Chang, T. *et al.* (2011) Brief report: phenotypic rescue of induced pluripotent stem cell-derived motoneurons of a spinal muscular atrophy patient. *Stem Cells* 29, 2090–2093
62. Yoshida, M. *et al.* (2015) Modeling the early phenotype at the neuromuscular junction of spinal muscular atrophy using patient-derived iPSCs. *Stem Cell Rep.* 4, 561–568
63. Ebert, A.D. *et al.* (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 457, 277–280
64. Kim, J.K. *et al.* (2017) Motor neuronal repletion of the NMJ organizer, Agrin, modulates the severity of the spinal muscular atrophy disease phenotype in model mice. *Hum. Mol. Genet.* 26, 2377–2385
65. Tejero, R. *et al.* (2020) R-Roscovitine improves motoneuron function in mouse models for spinal muscular atrophy. *iScience* 23, 100826
66. Villalon, E. *et al.* (2019) AAV9-Stathmin1 gene delivery improves disease phenotype in an intermediate mouse model of spinal muscular atrophy. *Hum. Mol. Genet.* 28, 3742–3754
67. Rehorst, W.A. *et al.* (2019) Muscle regulates mTOR dependent axonal local translation in motor neurons via CTRP3 secretion: implications for a neuromuscular disorder, spinal muscular atrophy. *Acta Neuropathol. Commun.* 7, 154
68. Kaifer, K.A. *et al.* (2019) AAV9-mediated delivery of miR-23a reduces disease severity in Smn2B/-SMA model mice. *Hum. Mol. Genet.* 28, 3199–3210
69. Kaifer, K.A. *et al.* (2017) Plastin-3 extends survival and reduces severity in mouse models of spinal muscular atrophy. *JCI Insight* 2, e89970
70. Hosseinibarkooie, S. *et al.* (2016) The power of human protective modifiers: PLS3 and CORO1C unravel impaired endocytosis in spinal muscular atrophy and rescue SMA phenotype. *Am. J. Hum. Genet.* 99, 647–665
71. Riessland, M. *et al.* (2017) Neurocalcin delta suppression protects against spinal muscular atrophy in humans and across species by restoring impaired endocytosis. *Am. J. Hum. Genet.* 100, 297–315

72. Zhou, H. *et al.* (2020) Myostatin inhibition in combination with antisense oligonucleotide therapy improves outcomes in spinal muscular atrophy. *J. Cachexia. Sarcopenia Muscle* 11, 768–782
73. Kim, J.K. *et al.* (2020) Muscle-specific SMN reduction reveals motor neuron-independent disease in spinal muscular atrophy models. *J. Clin. Invest.* 130, 1271–1287
74. Hardiman, O. *et al.* (2017) Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Primers* 3, 17071
75. Ling, S.C. *et al.* (2013) Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 79, 416–438
76. van Rheenen, W. *et al.* (2016) Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1043–1048
77. Bozzoni, V. *et al.* (2016) Amyotrophic lateral sclerosis and environmental factors. *Funct. Neurol.* 31, 7–19
78. Mejzini, R. *et al.* (2019) ALS genetics, mechanisms, and therapeutics: where are we now? *Front. Neurosci.* 13, 1310
79. Moloney, E.B. *et al.* (2014) ALS as a distal axonopathy: molecular mechanisms affecting neuromuscular junction stability in the presymptomatic stages of the disease. *Front. Neurosci.* 8, 252
80. Loeffler, J.P. *et al.* (2016) The role of skeletal muscle in amyotrophic lateral sclerosis. *Brain Pathol.* 26, 227–236
81. Dadon-Nachum, M. *et al.* (2011) The "dying-back" phenomenon of motor neurons in ALS. *J. Mol. Neurosci.* 43, 470–477
82. Cantor, S. *et al.* (2018) Preserving neuromuscular synapses in ALS by stimulating MuSK with a therapeutic agonist antibody. *Elife* 7, e34375
83. Zu, T. *et al.* (2013) RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proc. Natl. Acad. Sci. U. S. A.* 110, E4968–E4977
84. Shi, Y. *et al.* (2018) Haploinsufficiency leads to neurodegeneration in C9ORF72 ALS/FTD human induced motor neurons. *Nat. Med.* 24, 313–325
85. Liu, Y. *et al.* (2016) C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. *Neuron* 90, 521–534
86. Cykowski, M.D. *et al.* (2019) Dipeptide repeat (DPR) pathology in the skeletal muscle of ALS patients with C9ORF72 repeat expansion. *Acta Neuropathol.* 138, 667–670
87. Lynch, E. *et al.* (2019) C9ORF72-related cellular pathology in skeletal myocytes derived from ALS-patient induced pluripotent stem cells. *Dis. Model. Mech.* 12, dmm039552
88. Melamed, Z. *et al.* (2019) Premature polyadenylation-mediated loss of stathmin-2 is a hallmark of TDP-43-dependent neurodegeneration. *Nat. Neurosci.* 22, 180–190
89. Vogler, T.O. *et al.* (2018) TDP-43 and RNA form amyloid-like myo-granules in regenerating muscle. *Nature* 563, 508–513
90. Weihl, C.C. *et al.* (2008) TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* 79, 1186–1189
91. Alami, N.H. *et al.* (2014) Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron* 81, 536–543
92. Gasset-Rosa, F. *et al.* (2019) Cytoplasmic TDP-43 de-mixing independent of stress granules drives inhibition of nuclear import, loss of nuclear TDP-43, and cell death. *Neuron* 102, 339–357 e7
93. Orr, B.O. *et al.* (2020) Presynaptic homeostasis opposes disease progression in mouse models of ALS-like degeneration: evidence for homeostatic neuroprotection. *Neuron* 107, 95–111
94. Mathis, S. *et al.* (2019) Genetics of amyotrophic lateral sclerosis: a review. *J. Neurol. Sci.* 399, 217–226
95. Sharma, A. *et al.* (2016) ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. *Nat. Commun.* 7, 10465
96. Lopez-Erauskin, J. *et al.* (2018) ALS/FTD-linked mutation in FUS suppresses intra-axonal protein synthesis and drives disease without nuclear loss-of-function of FUS. *Neuron* 100, 816–830 e7
97. So, E. *et al.* (2018) Mitochondrial abnormalities and disruption of the neuromuscular junction precede the clinical phenotype and motor neuron loss in hFUSWT transgenic mice. *Hum. Mol. Genet.* 27, 463–474
98. Picchiarelli, G. *et al.* (2019) FUS-mediated regulation of acetylcholine receptor transcription at neuromuscular junctions is compromised in amyotrophic lateral sclerosis. *Nat. Neurosci.* 22, 1793–1805
99. Arnold, F.J. and Merry, D.E. (2019) Molecular mechanisms and therapeutics for SBMA/Kennedy's disease. *Neurotherapeutics* 16, 928–947
100. Soraru, G. *et al.* (2008) Spinal and bulbar muscular atrophy: skeletal muscle pathology in male patients and heterozygous females. *J. Neurol. Sci.* 264, 100–105
101. Al-Sarraj, S. *et al.* (2014) Mitochondrial abnormalities and low grade inflammation are present in the skeletal muscle of a minority of patients with amyotrophic lateral sclerosis; an observational myopathology study. *Acta Neuropathol. Commun.* 2, 165
102. Lombardi, V. *et al.* (2019) Muscle and not neuronal biomarkers correlate with severity in spinal and bulbar muscular atrophy. *Neurology* 92, e1205–e1211
103. Khalil, M. *et al.* (2018) Neurofilaments as biomarkers in neurological disorders. *Nat. Rev. Neurol.* 14, 577–589
104. Lieberman, A.P. *et al.* (2014) Peripheral androgen receptor gene suppression rescues disease in mouse models of spinal and bulbar muscular atrophy. *Cell Rep.* 7, 774–784
105. Cortes, C.J. *et al.* (2014) Muscle expression of mutant androgen receptor accounts for systemic and motor neuron disease phenotypes in spinal and bulbar muscular atrophy. *Neuron* 82, 295–307
106. Larsson, L. *et al.* (2019) Sarcopenia: aging-related loss of muscle mass and function. *Physiol. Rev.* 99, 427–511
107. Gonzalez-Freire, M. *et al.* (2014) The neuromuscular junction: aging at the crossroad between nerves and muscle. *Front. Aging Neurosci.* 6, 208
108. Kwon, Y.N. and Yoon, S.S. (2017) Sarcopenia: neurological point of view. *J. Bone Metab.* 24, 83–89
109. Fish, L.A. and Fallon, J.R. (2020) Multiple MuSK signaling pathways and the aging neuromuscular junction. *Neurosci. Lett.* 135014
110. Chai, R.J. *et al.* (2011) Striking denervation of neuromuscular junctions without lumbar motoneuron loss in geriatric mouse muscle. *PLoS One* 6, e28090
111. Steffl, M. *et al.* (2017) Relationship between sarcopenia and physical activity in older people: a systematic review and meta-analysis. *Clin. Interv. Aging* 12, 835–845
112. Fujimori, K. *et al.* (2018) Modeling sporadic ALS in iPSC-derived motor neurons identifies a potential therapeutic agent. *Nat. Med.* 24, 1579–1589
113. Ko, C.P. and Robitaille, R. (2015) Perisynaptic Schwann cells at the neuromuscular synapse: adaptable, multitasking glial cells. *Cold Spring Harb. Perspect. Biol.* 7, a020503
114. Arbour, D. *et al.* (2017) New perspectives on amyotrophic lateral sclerosis: the role of glial cells at the neuromuscular junction. *J. Physiol.* 595, 647–661
115. Santosa, K.B. *et al.* (2018) Clinical relevance of terminal Schwann cells: an overlooked component of the neuromuscular junction. *J. Neurosci. Res.* 96, 1125–1135
116. Barik, A. *et al.* (2016) Schwann cells in neuromuscular junction formation and maintenance. *J. Neurosci.* 36, 9770–9781
117. Doerflinger, N.H. *et al.* (2003) Inducible site-specific recombination in myelinating cells. *Genesis* 35, 63–72
118. Reddy, L.V. *et al.* (2003) Glial cells maintain synaptic structure and function and promote development of the neuromuscular junction *in vivo*. *Neuron* 40, 563–580
119. Bruneteau, G. *et al.* (2015) Endplate denervation correlates with Nogo-A muscle expression in amyotrophic lateral sclerosis patients. *Ann. Clin. Transl. Neurol.* 2, 362–372
120. Son, Y.J. and Thompson, W.J. (1995) Schwann cell processes guide regeneration of peripheral axons. *Neuron* 14, 125–132
121. Schaeffer, L. *et al.* (2001) Targeting transcription to the neuromuscular synapse. *Neuron* 31, 15–22
122. Sahoo, P.K. *et al.* (2018) Axonal mRNA transport and translation at a glance. *J. Cell Sci.* 131
123. Holt, C.E. and Schuman, E.M. (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* 80, 648–657

124. Chevrel, G. *et al.* (2006) The role of neurotrophins in muscle under physiological and pathological conditions. *Muscle Nerve* 33, 462–476
125. Feng, Z. and Ko, C.P. (2008) Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-beta1. *J. Neurosci.* 28, 9599–9609
126. Schmidt, B.J. *et al.* (2002) Expression of X-linked bulbospinal muscular atrophy (Kennedy disease) in two homozygous women. *Neurology* 59, 770–772
127. Ranganathan, S. *et al.* (2009) Mitochondrial abnormalities in spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* 18, 27–42
128. Borgia, D. *et al.* (2017) Increased mitophagy in the skeletal muscle of spinal and bulbar muscular atrophy patients. *Hum. Mol. Genet.* 26, 1087–1103
129. Giorgetti, E. *et al.* (2016) Rescue of metabolic alterations in AR113Q skeletal muscle by peripheral androgen receptor gene silencing. *Cell Rep.* 17, 125–136
130. Chua, J.P. *et al.* (2014) Transcriptional activation of TFEB/ZKSCAN3 target genes underlies enhanced autophagy in spinobulbar muscular atrophy. *Hum. Mol. Genet.* 23, 1376–1386
131. Rusmini, P. *et al.* (2015) Aberrant autophagic response in the muscle of a knock-in mouse model of spinal and bulbar muscular atrophy. *Sci. Rep.* 5, 15174
132. Poort, J.E. *et al.* (2016) Neuromuscular junctions are pathological but not denervated in two mouse models of spinal bulbar muscular atrophy. *Hum. Mol. Genet.* 25, 3768–3783
133. Halievski, K. *et al.* (2015) Androgen-dependent loss of muscle BDNF mRNA in two mouse models of SBMA. *Exp. Neurol.* 269, 224–232