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REVIEW

Axonal Excitability in Amyotrophic Lateral Sclerosis

Axonal Excitability in ALS

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Abstract Axonal excitability testing provides *in vivo* assessment of axonal ion channel function and membrane potential. Excitability techniques have provided insights into the pathophysiological mechanisms underlying the development of neurodegeneration and clinical features of amyotrophic lateral sclerosis (ALS) and related neuromuscular disorders. Specifically, abnormalities of Na^+ and K^+ conductances contribute to development of membrane hyperexcitability in ALS, thereby leading to symptom generation of muscle cramps and fasciculations, in addition to promoting a neurodegenerative cascade via Ca^{2+}-mediated processes. Modulation of axonal ion channel function in ALS has resulted in significant symptomatic improvement that has been accompanied by stabilization of axonal excitability parameters. Separately, axonal ion channel dysfunction evolves with disease progression and correlates with survival, thereby serving as a potential therapeutic biomarker in ALS. The present review provides an overview of axonal excitability techniques and the physiological mechanisms underlying membrane excitability, with a focus on the role of axonal ion channel dysfunction in motor neuron disease and related neuromuscular diseases.

Keywords Amyotrophic lateral sclerosis · axonal excitability · hyperexcitability · neuromuscular disorders · ion channels · neurodegeneration

Introduction

Axonal excitability techniques provide *in vivo* assessment of axonal membrane and ion channel function, yielding complementary information to conventional nerve conduction studies [1, 2]. Excitability techniques have been utilized across the spectrum of neurologic disorders, providing invaluable insights into the pathophysiologic processes underlying a host of neuromuscular disorders [3]. Specifically, in motor neuron disease, also termed amyotrophic lateral sclerosis (ALS), axonal excitability techniques have provided vital information on axonal ion channel dysfunction, which has been of pathophysiologic significance [4]. Importantly, abnormalities of axonal Na^+ and K^+ conductances have been consistently identified in ALS [5–10], resulting in symptom generation such as muscle cramps and fasciculations, as well as serving as potential therapeutic targets [11]. Similarly, axonal excitability parameters have yielded critical pathophysiological insights in ALS-mimicking disorders [3, 12–15] Table 1.

Importantly, the potential use of excitability techniques as a biomarker to provide information about axonal function relevant to the development of disease, disease progression, and treatment response may prove to be a useful endpoint in clinical trials, particularly in monitoring the effects of therapeutic agents. In this review article, we provide an overview of the physiological mechanisms underlying axonal excitability, and focus on the importance of axonal ion channel dysfunction as a pathogenic and prognostic biomarker in ALS and related neuromuscular diseases.

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Table 1 Peripheral axonal excitability findings in neuromuscular disorders

Disease	Excitability findings			References
	Strength-duration time constant	Recovery cycle	Threshold electrotonus and I/V relationship	
Motor neuron disease	Prolonged	Increased superexcitability	Increased TEd and TEh	[6–8, 58, 61, 76]
Kennedy's disease	Prolonged	Minor changes	Increased hyperpolarizing I/V slope; increased TEd	[116]
Multifocal motor neuropathy	No change	Decreased RRP; increased superexcitability	Increased TEd and TEh; decreased resting I/V slope	[14, 139, 140]
Chronic inflammatory demyelinating polyneuropathy	Reduced	Variability with disease stage—decreased recovery cycle parameters or no changes	Variability with disease stage—increased TEd and TEh or no changes	[140, 142, 144, 145]
Spinal muscular atrophy	Prolonged in severe disease	Increased subexcitability in severe disease	Increased TEd and TEh; decreased resting I/V slope in severe disease	[12, 61]
Acquired neuromyotonia	No change	Increased subexcitability or no change	No change	[15, 135]
Benign cramp fasciculation syndrome	No change	No change	No changes in conventional measures; decreased TEh at −70 and −100%	[136, 137]

I/V = current threshold relationship; TEh = threshold electrotonus (hyperpolarizing direction); TEd = threshold electrotonus (depolarizing direction); RRP = relative refractory period

The Clinical Assessment of Axonal Excitability

The availability and utility of axonal excitability techniques have progressed markedly over the last 20 years, with the advent of commercialization of equipment and specialized software. In contrast to monitoring maximal compound amplitudes, as occurs with conventional nerve conduction studies, excitability studies examine the function of constituent axonal conductances, assessing changes in current required to produce a constant compound muscle action potential (CMAP) response. Accordingly, excitability studies provide information about axonal function, membrane potential, and ion channel properties (Fig. 1). In clinically applied protocols, the constant response method of assessing excitability is typically utilized [2]. Threshold tracking relies on the definition of "threshold" as the stimulus strength required to produce a CMAP response of a specified target amplitude. In threshold tracking protocols, the current required to produce the target response (threshold) is tracked online, with the stimulus current altered proportionally to the discrepancy between the target and actual response [9]. Software enabling semiautomated threshold tracking has been utilized in axonal excitability protocols via the QTracS program (Institute of Neurology, UK). The modern TROND protocol was developed by Kiernan, Burke, and Bostock [16] during the 1999 Nordic course in clinical axonal electrophysiology held in Trondheim, and provides a package of excitability techniques for assessing motor nerve excitability, while sensory nerve excitability protocols were subsequently developed [17]. Interpretation of axonal excitability studies can be complex, owing to the multifactorial nature of changes in ionic conductances, as well as passive and active membrane properties. Accordingly, the development of a mathematical model of the excitability properties of human axons has assisted in interpretation of axonal excitability changes [18–20].

The key axonal excitability parameters include threshold, strength-duration time constant, rheobase, threshold electrotonus, recovery cycle, and current/threshold relationship (Fig. 2). These protocols rely on an accurate stimulus response curve in which the current is progressively increased from zero until the CMAP amplitude is supramaximal, producing a sigmoid curve.

Threshold reflects the amount of stimulus current required to activate an axon and produce a CMAP of a specific amplitude, usually 40% of the maximal CMAP amplitude [1, 2]. The threshold is related to membrane potential, as membrane hyperpolarization increases and depolarization decreases the threshold. Additional factors such as nerve ischemia may also impact on threshold, and, consequently, assessment of axonal

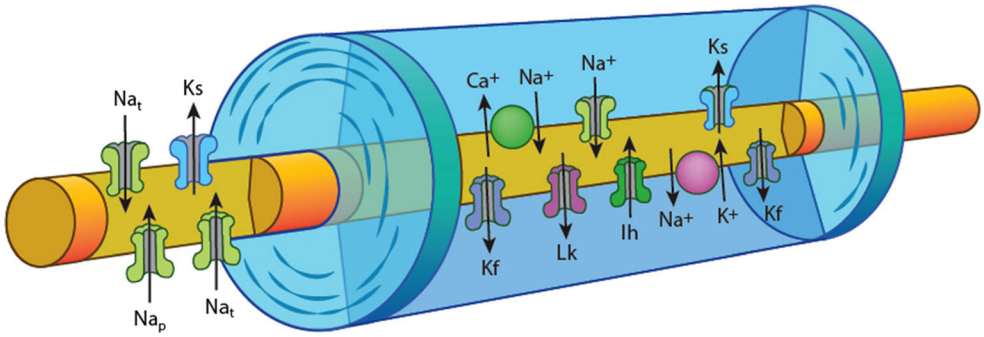

Fig. 1 Schematic of axon structure and ion channel distribution, demonstrating the node of Ranvier with high density of voltage-gated Na^+ channels [both transient (Na_t) and persistent (Na_p)] and slow K^+ channels (K_s). The internode under the myelin sheath is depicted with fast K^+ channels (K_f) located adjacent to the node, the hyperpolarization activated cation conductance (I_h), slow K^+ channels, and voltage-independent leak conductances (Lk) along with the energy dependent Na^+/K^+ pump and the Na^+/Ca^{2+} exchanger

excitability parameters (discussed later) is required to provide a clear depiction of membrane excitability [21–25].

The *strength-duration time constant* (τ_{SD}), also termed chronaxie, is a membrane time constant, assessing the relationship between stimulus strength and duration [26–28]. τ_{SD} measures the rate at which the threshold current declines as the stimulus duration is increased and can be calculated using the ratio between two different stimulus durations according to Weiss' law (Fig. 2a) [29]. Rheobase is defined as the minimum current strength (mA) required to produce a response for a stimulus of infinite duration [1, 7]. Both τ_{SD} and rheobase are nodal properties dependent on passive membrane properties and persistent Na^+ channel conductances (I_{NaP}) [30].

The persistent Na^+ current represents a fraction of the total Na^+ current (approximately 1–2% of total current) and does not inactivate, therefore influencing membrane excitability [30–32]. Voltage-gated Na^+ channels are composed of 1 alpha (α) and 4 beta (β1–4) subunits [33, 34], with the α subunits organized in 4 homologous domains (I–IV), consisting of 6

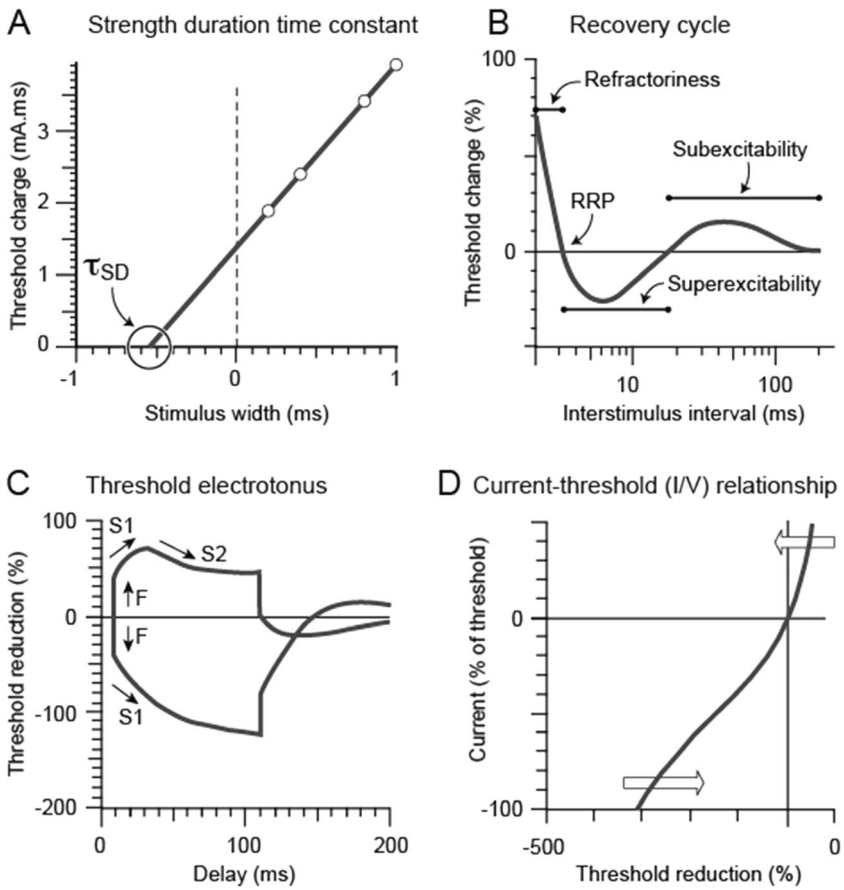

Fig. 2 **a** Strength-duration time constant (τsd) plotted as the negative intercept on the *x*-axis of the linear relationship between stimulus width and threshold charge. **b** Recovery cycle plotted with the relative refractory period, superexcitability, and subexcitability depicted on the figure. **c** Threshold electrotonus in response to polarizing currents set to ± 40% of threshold. The initial response to polarization (F phase) is followed by a slow change in threshold (S1 phase), with threshold increase in the hyperpolarizing direction (plotted downwards) and threshold decrease in the depolarizing direction (plotted upwards), followed by accommodation to depolarization (S2 phase). **d** Current threshold (I/V) relationship plotted with response to depolarizing current in the upper right quadrant and response to hyperpolarizing current in the lower left quadrant

transmembrane α helices (S1–S6) and a selectivity filter located between S5 and S6 segments. Na^+ channel inactivation is mediated by an intracellular loop connecting domains III and IV, which blocks the pore from inside during continuous membrane depolarization. A total of 10 Na^+ channel isoforms have been identified (Na_v 1.1–Na_v 1.9 and Na_x [33–35]), and the Na_v1.6 isoform expressed at the nodes of Ranvier is thought to produce I_{NaP} [33–36].

Changes in the τ_{SD} and rheobase may be influenced by multiple factors—with changes in resting membrane potential, nerve geometry, and structure, such as axonal loss or demyelination, and discrete changes in nodal Na^+ conductances producing modification of membrane excitability [37–39].

The *recovery cycle of excitability* (Fig. 2b) determines the profile of excitability changes following an action potential [1]. Utilizing paired pulses, the interstimulus interval is progressively altered between 2 ms and 200 ms, revealing a characteristic pattern of excitability changes [40]. When the interstimulus interval is short (<4 ms), the current required to produce a subsequent action potential is increased [40]. The axon is completely refractory for a period of 0.5 to 1 ms during which no action potentials can be generated, owing to the inactivation of transient voltage-gated Na^+ channels, termed the *absolute refractory period* [21]. Following this absolute refractory period, there is development of the *relative refractory period*, lasting up to 4 ms (Fig. 2b), during which time action potential generation is possible but more difficult as Na^+ channels recover from inactivation [41]. The extent of the relative refractory period can be measured as an increase in current required to generate a CMAP (termed refractoriness) [41, 42].

Transient Na^+ channel kinetics, and, accordingly, refractoriness, may be influenced by changes in membrane potential, whereby membrane depolarization increases the extent of Na^+ channel inactivation and refractoriness, and hyperpolarization reduces refractoriness by reducing the degree of inactivated Na^+ channels [1]. Na^+ channel inactivation and refractoriness are also sensitive to temperature changes, and reduction in limb temperature markedly increases refractoriness [43, 44].

The next phase of the recovery cycle has been termed *superexcitability* (lasting 15 ms and peaking between 5 and 7 ms; Fig. 2b), reflecting enhanced membrane excitability due to depolarizing afterpotential formation [1, 40, 45, 46]. Superexcitability appears mediated by re-excitation of the nodal membrane produced by the depolarizing afterpotential, leading to discharge of current stored on the internodal membrane following an action potential (akin to capacitive charging of the internode). The amplitude and time course of the afterpotential is limited by activation of fast paranodal K^+ channels [47]. Changes in membrane potential influence superexcitability, with membrane depolarization reducing superexcitability through activation of fast K^+ channels [45], and hyperpolarization increasing superexcitability [48].

The final phase of the recovery cycle has been termed *subexcitability*, a period of reduced axonal excitability lasting approximately 100 ms (Fig. 2b). Subexcitability is due to reduced membrane excitability due to the slow activation of K^+ channels [46]. Membrane depolarization increases subexcitability, via changes to the electrochemical gradient for K^+. However, conversely, membrane depolarization secondary to increases in extracellular K^+ concentration, as occurs with ischemia or renal failure, results in subexcitability reduction [48–50].

Threshold Electrotonus (TE) describes the changes in threshold produced by long-lasting subthreshold currents, providing insight into both nodal and internodal membrane conductances (Fig. 2c) [1, 2, 51]. TE utilizes a 100-ms subthreshold current pulse with sequential depolarizing and hyperpolarizing currents set to +20% or 40% and −20 or 40% of the threshold current [4]. The changes in the threshold current can be measured at varying time intervals to reveal the excitability profile in response to subthreshold conditioning currents [16, 17].

In response to depolarizing currents, threshold is immediately reduced until mitigated by the activity of slow K^+ channels which accommodate the effect of depolarization [51]. The initial fast response (F phase; Fig. 3c) reflects rapid changes in membrane threshold at the node. This is followed by slower changes in threshold over tens of milliseconds with depolarizing current, termed the S1 phase, reflecting the spread of current to the internodal membrane and peaking 20 ms after the onset of the current pulse. After this threshold begins to return to baseline, termed the S2 phase, reflecting activation of nodal and internodal slow K^+ channels [2, 51].

With hyperpolarizing current pulses, threshold is increased proportional to the current pulse (F phase). Subsequent closure of K^+ channels with hyperpolarization further increases threshold [2]. During the S1 phase, further hyperpolarization occurs, peaking at 100 to 150 ms after the onset of the subthreshold conditioning current and returning to baseline. After prolonged hyperpolarization, the S3 phase commences as a result of activation of the hyperpolarizing-activated cation conductance (I_h) [1–3, 52]. Activation of I_h following prolonged hyperpolarization attenuates the increase in threshold [52] and limits the extent of hyperpolarization in response to high-frequency activity [46]. As high-frequency activity can induce conduction failure in axons with a reduced safety factor of transmission, the I_h current may be critical in preventing conduction failure [53]. Termination of subthreshold conditioning currents results in overshoot of depolarizing and hyperpolarizing thresholds, mediated by

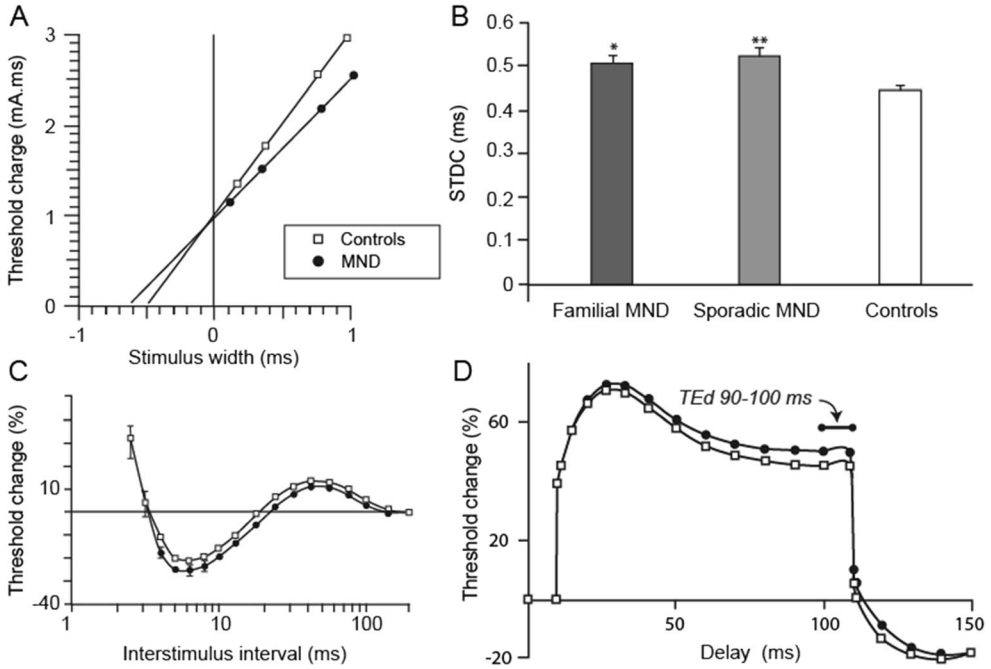

Fig. 3 **a** The strength-duration time constant reflects nodal persistent Na^+ channel conductances and was significantly increased in patients with amyotrophic lateral sclerosis (ALS; filled circles) compared to controls (clear squares). **b** Mean SDTC was significantly increased in both familial ALS and sporadic ALS compared with controls (*$p < 0.05$; **$p < 0.01$). **c** Recovery cycle waveforms in patients with motor neuron disease (MND; filled circles) compared with controls (clear squares) demonstrating increased superexcitability in patients with ALS. **d** Threshold electrotonus waveforms in patients with ALS (filled circles) compared with controls (clear squares) demonstrating increased threshold change in threshold electrotonus depolarizing direction (TEd) 90–100 ms in patients with ALS

deactivation of slow K^+ channels, and the slow kinetics of I_h [1].

As with other axonal excitability parameters, TE is influenced by changes in membrane potential. Specifically, membrane depolarization activates K^+ channels, resulting in reduction of the S1 phase in both depolarizing and hyperpolarizing directions, causing a "fanning in" appearance of TE [1, 2, 48]. Conversely, hyperpolarization closes K^+ channels, increasing both S1 and S2 phases and producing a "fanning out" appearance of TE [1, 2, 48].

The *current threshold relationship (I/V relationship)* utilizes a range of depolarizing and hyperpolarizing current strengths over long-duration subthreshold 200-ms pulses [48]. Currents are applied from +50% (depolarizing) to −100% (hyperpolarizing) of the control threshold in 10% steps (Fig. 2d) [16, 17]. The I/V relationship estimates rectifying properties of both nodal and internodal axonal segments [2]. Accommodation to depolarization occurs via fast and slow K^+ channel activation, termed outward rectification [54]. While slow K^+ channels are located at higher density at the node and fast K^+ channels are located at the juxtaparanode adjacent to the node, both act broadly to reduce excitability following impulse conduction [55]. With hyperpolarizing currents, inward rectification occurs owing to the activation of I_h [52].

Axonal Excitability Changes in ALS

Disturbances in membrane excitability and axonal ion-channel function have been identified from the earliest application of axonal excitability techniques in patients with ALS [5]. Subsequently, numerous studies have reported axonal dysfunction in patients with ALS characterized by prolonged τ_{SD}, increased superexcitability, and abnormalities of threshold electrotonus [6–8, 56–58]. These changes have been specifically attributed to aberrant ionic conductances, particularly increased Na^+ and decreased axonal K^+ conductances, a profile that may underlie neurodegeneration and contribute to symptoms, such as fasciculations and muscle cramps [59].

Prolongation of τ_{SD} has been consistently identified in sporadic ALS and linked to neurodegeneration (Fig. 3a) [6, 7, 56, 60–63].In addition, prolongation of τ_{SD} has been identified in atypical ALS phenotypes, such as the flail arm variant [64], as well as familial forms of ALS linked to mutations in *SOD1* and *c9orf72* (Fig. 3b) [9, 56, 65]. The increase of τ_{SD} was most prominent in patients with ALS with a moderate degree of lower motor neuron dysfunction, suggesting that axonal hyperexcitability is an early feature in ALS [8]. Interestingly, the split-hand phenomenon [66–68], a specific sign in ALS characterized by preferential wasting of the abductor pollicis brevis muscle, was reported to be associated with greater increases in the τ_{SD} in median motor nerves [63, 69].

Upregulation of persistent Na^+ conductances appears to underlie the prolongation of τ_{SD} in ALS [60, 70]. In addition, a reduction in fast and slow K^+ channel conductances has also been proposed as a contributing mechanism in prolongation of τ_{SD} by inducing membrane depolarization [8]. These changes in axonal excitability have been linked to axonal degeneration and development of symptoms such as fasciculations and muscle cramps [8, 10, 56]. Recent studies have suggested that the increase in axonal excitability was an adaptive and potentially neuroprotective mechanisms [71]. This seems unlikely given that treatment with Na^+ blocking agents, flecainide and mexiletine (discussed below), was not associated with a more rapid progression of the disease [11, 72, 73]. Importantly, τ_{SD} appears to be a robust biomarker of survival in ALS [74], whereby a prolonged τ_{SD} was associated with a significantly shorter survival, further underscoring the pathogenic basis of axonal hyperexcitability in ALS [80]. Similarly, rapid functional decline in motor performance has also been linked to prolonged τ_{SD} [75], further suggesting a link between excitability and clinical progression.

In concert with prolonged τ_{SD}, changes in threshold electrotonus have been identified in sporadic ALS, although the pattern of changes appears heterogeneous [5, 6, 8, 76, 77]. Specifically, a "fanned out" appearance of TE, whereby greater threshold changes were evident with depolarizing and hyperpolarizing subthreshold currents, has been documented in ALS [6, 77]. In contrast, others have documented greater changes only in response to subthreshold depolarizing currents (Fig. 3d) [5, 6]. A sudden reduction of membrane excitability in conjunction with an abrupt increase in threshold has also been reported in ALS [5], although such changes appear to be an infrequent finding [4].

TE changes in ALS evolve with disease progression, as evidenced by an increase in depolarizing TE with longitudinal follow-up over 3 months [58]. The longitudinal changes in TE were accompanied by axonal degeneration and functional decline, thereby implying a pathogenic role for TE changes in ALS. In addition, "pseudonormalization" of TE changes has also been reported with disease progression and associated with axonal degeneration [4], with the most hyperexcitable axons degenerating with advanced disease. Underscoring this notion is the greater variability of axonal excitability at different target amplitudes, with preferential hyperexcitability evident in low-threshold axons [78]. Importantly, these longitudinal changes in TE could account for the variability reported in previous axonal excitability studies in ALS [5, 78], and suggest that targeting the "hyperexcitable" axons early in the disease process could prove therapeutically useful. Separately, the TE changes in ALS appear to be more prominent in distal axonal segments [79], suggesting a propensity for axonal hyperexcitability to develop distally, and potentially explaining the notion that fasciculations are generated distally in the axon [59, 80]. A similar pattern of TE changes were reported in *SOD1* and *c9orf72* familial ALS, and were linked to neurodegeneration [9, 56]. In contrast, no significant TE changes were evident in asymptomatic mutation (*SOD1* and *c9orf72*) carriers, suggesting that factors other than inheritance of genetic mutations are required for the development of such excitability changes in ALS.

In addition to changes in TE, significant abnormalities of the recovery cycle of excitability have also been reported in sporadic ALS. Specifically, an increase in superexcitability has accompanied changes in TE (Fig. 3c) [6], although this has not been a consistent finding [9], further underscoring the heterogeneity of the disease process. In part, this heterogeneity may develop in the context of dying and degenerating axons. Importantly, the increase in superexcitability appears more prominent with disease progression and is also linked to functional decline [58]. Interestingly, increased superexcitability was reported in *SOD1*- but not *c9orf72*-associated familial ALS [9, 10, 56]. As with TE, asymptomatic mutation carriers exhibited a normal level of superexcitability, underscoring the notion that axonal ion channel dysfunction develops following disease onset in familial ALS.

Mathematical modeling studies have suggested that reduction of nodal and internodal slow K^+ conductances underlies the abnormalities of TE in sporadic and familial ALS cohorts [8]. Of further relevance, techniques utilizing double conditioning pulses during the recovery cycle have provided supporting evidence of impairment of slow K^+ conductances in ALS [81]. Separately, increases of superexcitability in ALS reflect dysfunction of paranodal fast K^+ channels [4]. Support for such an explanation has been provided by gene expression studies documenting reduced mRNA expression of K^+ channel genes, both paranodal fast K^+ channel genes (*KCNA1* and *KCNA2*) and the nodal K^+ channel gene (*KCNQ2*), in spinal motor neurons of patients with ALS [82]. Of further relevance, immunohistochemical studies have disclosed reduced axonal expression of K^+ channels in patients with sporadic ALS [83].

Pathophysiological Basis of Excitability Changes in ALS

Given that fasciculations and muscle cramps are prominent features of ALS that reflect ectopic activity in motor axons [59, 80, 84], abnormalities of K^+ and Na^+ ion channel function in ALS is likely to underlie the generation of such symptoms. The reduction of axonal K^+ currents would decrease the tendency to membrane hyperpolarization, while upregulation of persistent Na^+ conductances would increase the depolarizing drive, leading to membrane hyperexcitability [85]. Importantly, reduction of K^+ currents has been associated with the generation of fasciculations in ALS [5], while axonal hyperexcitability was induced by presynaptic blockade of K^+ channels [86]. In addition, blockade of persistent Na^+ conductances with mexiletine led to a dose-dependent reduction in

muscle cramp frequency and severity [11], further underscoring the clinical importance of the axonal excitability changes in ALS.

Of further relevance, the changes in axonal ion channel function are associated with neurodegeneration in ALS [4, 87]. Although the precise mechanisms by which the axonal excitability changes lead to neurodegeneration remain to be elucidated, it has been postulated that an influx of Na^+ ions results in reverse operation of the Na^+/Ca^{2+} exchanger, with the resultant accumulation of intra-axonal accumulation of Ca^{2+} ions and, ultimately, activation of Ca^{2+}-dependent enzyme pathways leading to motor neuron degeneration [88–91].Support for such a mechanism was provided by transgenic ALS mouse model studies disclosing a global reduction of Na^+/K^+-ATPase activity [92], leading to an increase in intracellular Na^+ ion concentration [89], and thereby resulting in Ca^{2+}-mediated neurodegeneration. Importantly, expression of the functional Na^+/K^+ ATPase appears to be neuroprotective in transgenic mouse models, while reduced expression was documented in the spinal cord of patients with sporadic and familial ALS [92]. Of further relevance, axons in the $SOD1^{G127X}$ mouse model appear to be less resistant to activity-induced changes in intracellular ion concentrations and degeneration [93], underscoring the pathogenic importance of Na^+/K^+ ATPase dysfunction in ALS. In addition, impairment of Na^+/K^+ ATPase activity along with altered expression was reported in the spinal cords of $SOD1^{G93A}$ mice and patients with ALS [94], and was associated with accelerated neuronal degeneration in the mouse model [94]. In contrast, the function of Na^+/K^+ ATPase was reportedly normal in patients with ALS, when measured from peripheral motor axons, and was associated with the development of fatigue [95]. This apparent discordance could be explained by disease heterogeneity, with the surviving axons demonstrating either normal pump function or a stronger reserve capacity of the Na^+/K^+ ATPase pump.

In addition to aberrant Na^+/K^+ ATPase activity, there is substantial evidence relating to the pathogenic role of hyperexcitability and aberrant persistent Na^+ conductances in ALS from transgenic mouse models [87]. Upregulation of persistent Na^+ currents was reported in cortical and spinal motoneurons in $SOD1^{G93A}$ mouse models [60, 96, 97], and appeared to be an early feature inhibited by riluzole [60], the only effective neuroprotective agent in ALS [98, 99]. Importantly, altered expression of specific voltage-gated Na^+ channel subunits, namely the β3 subunit, could underlie the upregulation of persistent Na^+ currents in ALS [100]. Of further relevance, increased persistent sodium currents and hyperexcitability were evident in brainstem motor neurons from $SOD1^{G93A}$ mice prior to onset of motor neuron degeneration [101]. In addition, noncell-autonomous toxicity, an important pathogenic process in ALS [87], appears to be mediated by astrocyte induced upregulation of persistent Na^+ conductances leading to increased hyperexcitability and intracellular Ca^{2+} ion concentration [102]. Importantly, reduction of persistent Na^+ conductances by either specific (mexiletine) or nonspecific (spermidine and riluzole) agents inhibited hyperexcitability, normalized Ca^{2+} influx and prevented motoneuron degeneration [102], thereby suggesting a potential therapeutic role for Na^+ channel blocking agents in ALS.

Separately, pluripotent stem-cell techniques have provided additional support for the importance of axonal ion-channel dysfunction in ALS pathogenesis [103, 104]. Specifically, reduction in K^+ currents was reported in induced pluripotent stem cell-derived motor neurons from patients with ALS harboring *SOD1, C9orf72*, and *FUS* mutations [103]. The reduction in K^+ currents was associated with membrane hyperexcitability across the 3 genetic mutations, suggesting that hyperexcitability is a ubiquitous feature in ALS. Importantly, activation of voltage-gated K^+ channels by retigabine reduced hyperexcitability and improved *in vitro* survival [103]. Of further relevance, similar techniques have confirmed that neuronal hyperexcitability is an early feature in patients with ALS with *C9orf72* and *TARDBP* mutations, with hypoexcitability evident in advanced disease underpinned by a reduction in Na^+ and K^+ currents over time [104]. Taken together, these findings suggest that targeting the axonal ion channel function, especially in early stages of ALS, could be of therapeutic benefit.

A contrasting view suggested that neuronal hyperexcitability exerted neuroprotective effects in ALS [71, 105]. Specifically, enhancement of neuronal excitability in the transgenic *SOD1* mouse models, through activation of metabotropic cholinergic signaling and mammalian target of rapamycin pathways, appeared neuroprotective [71], while early neuronal hyperexcitability failed to induce neurodegeneration in a separate superoxide dismutase 1 mouse model [105]. Separately, neuronal hypoexcitability has been reported in motor neuronal cell lines derived from patients with ALS, and an increase in excitability by K^+ channel blockade appeared neuroprotective [106]. Separately, others have argued that neuronal excitability is not related to intrinsic axonal properties of motor neurons, but rather related to extrinsic factors [107]. Importantly, the suggestion the axonal excitability is neuroprotective has only been documented in transgenic mouse models, which are poor models of human ALS [108].

From a clinical trials perspective, axonal excitability studies have been utilized as potential biomarkers of therapeutic efficacy in ALS. Specifically, riluzole therapy was associated with partial normalization of superexcitability, while no significant changes were evident in TE or τ_{SD} [109]. In addition, excitability studies have also been utilized in clinical trials assessing the effects of Na^+ channel-blocking agents. Specifically, low-dose mexiletine (300 mg) did not exert any modulating effects on axonal excitability parameters, including τ_{SD}, potentially accounting for the absence of clinical

effectiveness [73]. Separately, axonal excitability studies disclosed stabilization of axonal ion channel function in patients with ALS treated with the Na^+ channel-blocking agent flecainide [72], associated with a reduced rate of lower motor neuron dysfunction. These clinical studies underscore the potential of utilizing axonal excitability parameters as biomarkers of therapeutic effectiveness in a drug trial setting. Currently two phase II trials assessing efficacy of retigabine (Clinicaltrials.gov ID: NCT02450552) and high-dose mexiletine (Clinicaltrials.gov ID: NCT01811355) in ALS are in progress where axonal excitability parameters are being utilized as secondary outcome measures.

Axonal Excitability in ALS Mimic Disorders

In terms of differential diagnoses of ALS, *Kennedy's disease*, also known as spinobulbomuscular atrophy, is often raised. Kennedy's disease is a slowly progressive inherited neurodegenerative disorder of motor and sensory neurons secondary to increased expansion of the polymorphic cytosine–adenine–guanine (CAG) repeat sequence (coding glutamine) in the androgen receptor gene on the X chromosome, at Xq11-12 [110, 111]. Clinically, Kennedy's disease is characterized by presence of generalized fasciculations, muscle cramps, fatigue, and postural hand tremor, followed by progressive muscle weakness, dysarthria, and dysphagia [110, 112].Facial fasciculations with pursing of the lips, called contraction fasciculations, are a specific feature of Kennedy's disease and may be evident in female heterozygote or homozygous mutation carriers [112, 113].

Conventional neurophysiologic studies in Kennedy's disease are characterized by generalized and persistent fasciculations [114]. Unlike ALS, the fasciculation potentials in Kennedy's disease are simple with lower frequency when compared with ALS [114]. In addition, the contraction fasciculations that are characteristic of Kennedy's disease, are electrically characterized by either myokymia-like discharges that are induced by facial movement or 20 to 40-Hz discharges of individual motor unit action potentials of 0.1 to several seconds' duration [115].

Axonal excitability studies in Kennedy's disease have disclosed marked abnormalities as indicated by prolonged τ_{SD}, greater changes in response to a subthreshold depolarizing currents and increased hyperpolarizing current/threshold gradient [116]. Importantly, the increase in the τ_{SD} was an early feature in Kennedy's disease and correlated with the fasciculation frequency, suggesting that upregulation of persistent Na^+ conductances was responsible for generation of symptoms, particularly fasciculations, and contributed to the neurodegeneration. The pathophysiological mechanisms by which the mutated androgen receptor induces axonal ion-channel dysfunction in Kennedy's disease remains to be elucidated, although transcriptional dysregulation is a likely mechanism [117]. Future studies assessing the effects of activating neuroprotective cellular processes, such as molecular chaperones, ubiquitin–proteasome system, and autophagy, on axonal excitability could provide critical pathogenic insights in Kennedy's disease.

Spinal muscular atrophy (SMA) is a pediatric disorder of the spinal motor neurons clinically characterized by development of muscle weakness and atrophy [12]. Mutations in the *SMN1* underlie the development of SMA with reduction in survival motor neuron protein levels forming the pathogenic basis of motor neuron degeneration in SMA [118]. While *SMN1* mutations may induce abnormalities in axonal and membrane transports, as well as axonal myelination [119], transgenic mouse model studies have identified membrane hyperexcitability of diseased motor neurons [120].

Axonal excitability studies in ambulatory patients with SMA identified reduction in motor amplitudes, without significant changes in axonal excitability parameters [12]. In contrast, prolongation of τ_{SD}, along with greater depolarizing TE changes and steeper changes in the early phase of hyperpolarizing TE, were reported in severely affected patients with SMA and correlated with disease severity. Mathematical modeling suggested that a combination of neuronal degeneration and regeneration, resulting in shorter internodal length, reduction of internodal fast K^+ currents, and an increase in nodal K^+ conductances, best accounted for the axonal excitability changes in SMA [12]. From a clinical perspective, changes in axonal excitability parameters, particularly in more advanced SMA, could serve as potential biomarkers of therapeutic effectiveness in future trials.

Acquired neuromyotonia (aNMT) refers to a group of autoimmune diseases characterized by continuous ectopic nerve activity, clinically manifesting with muscle cramps, fasciculations, and stiffness [121]. The neuromuscular symptoms may be accompanied by hyperhidrosis, sensory abnormalities, and central nervous system features [121], and voltage-gated K^+ channel-associated antibodies are evident in approximately 40% of patients [121, 122]. Conventional neurophysiologic studies in aNMT have identified the presence of spontaneous activity, including ongoing changes (positive sharp waves and fibrillation potentials), fasciculations, myokymia, multiple discharges, muscle cramps, and repetitive after-discharges after voluntary contraction [15, 122, 123]. Importantly, the fasciculation firing rate and frequency of doublet discharges were reportedly less frequent in aNMT when compared with ALS [124].

The site of generation of ectopic activity in aNMT remains to be elucidated, and some have suggested that the ectopic focus arises at the distal nerve terminal [15, 121, 123, 125–128]. In contrast, an ectopic focus arising at the level of the anterior horn cell or more centrally [129–131], has been proposed, with the latter hypothesis supported by the presence

central nervous system symptoms and inflammatory changes, including the presence of oligoclonal bands [121, 132, 133]. Separately, aNMT may resemble ALS, particularly in early disease stages [87], and rarely an "apparent" case of aNMT may progress to ALS [134]. Axonal excitability has provided critical pathophysiological insights in aNMT, disclosing largely normal axonal ion channel function [15, 135], and thereby suggesting that the ectopic focus originates at the motor nerve terminal. Importantly, despite the presence of ectopic activity, the axonal excitability findings were markedly different between aNMT and ALS, thereby further supporting the importance of axonal ion-channel dysfunction in ALS pathogenesis.

Further, benign-cramp fasciculation syndrome is a related condition characterized by fasciculations in the absence of other symptoms or disorders [124]. Patients with benign cramp-fasciculation syndrome demonstrated increased accommodation to extensive hyperpolarization, potentially due to greater I_h conductance [136]. This was not evident with conventional axonal excitability protocols, but only with stronger hyperpolarization (−70% and −100% of thresehold). In a small cohort of patients, a different pattern was identified with reduced accommodation in TE (hyperpolarizing direction; TEh) and TE (depolarizing direction; TEd), suggestive of reduced slow K^+ channel function [137], and indicating that there may be subgroups of patients with different membrane excitability.

Turning attention to *inflammatory neuropathies*, multifocal motor neuropathy (MMN) presents with limb-onset muscle atrophy, and is characterized by conduction block, fasciculations, and muscle cramps [138]. Axonal excitability studies in patients with MMN have demonstrated prominent changes in axonal membrane function, with a substantially different excitability profile when compared with ALS. Overall, the excitability changes were suggestive of membrane hyperpolarization distal to the site of conduction block [14, 139]. Specifically, the excitability profile was characterized by increased threshold change in depolarizing and hyperpolarizing TE, along with an increase in superexcitability. It was argued that the membrane hyperpolarization in MMN was due to excessive activation of the Na^+/K^+-ATPase in response to focal depolarization at the site of conduction block. Interestingly, reduction of τ_{SD} and an increase in rheobase were reported in MMN after intravenous immunoglobulin infusion (IVIg), a finding related to inhibition of persistent Na^+ conductances [140], and postulated as the mechanism of the neuroprotective effects of IVIg in MMN [141].

Significant abnormalities of axonal excitability have also been reported in chronic inflammatory demyelinating polyneuropathy (CIDP), an autoimmune inflammatory disorder of the sensory and motor nerves [140, 142, 143]. Specifically, CIDP can be associated with reduction of τ_{SD}, refractoriness, supernormality, and subexcitability, along with an increase in rheobase, findings attributed to the underlying pathology [140, 142, 144, 145]. However, variability in excitability between patients may reflect heterogenous clinical phenotypes. Importantly, IVIg therapy resulted in both short- and long-term partial normalization of axonal excitability, characterized by an increase in τ_{SD} and improvements in TE [144]. Consequently, axonal excitability testing may serve as a useful therapeutic biomarker of immunotherapy responsiveness in autoimmune neuropathies, and could guide more tailored treatment strategies for individual patients suffering with MMN and CIDP.

In conclusion, axonal excitability techniques have provided insights regarding nodal and internodal nerve function in ALS and related neuromuscular disorders. Importantly, axonal ion channel dysfunction, characterized by abnormalities of Na^+ and K^+ conductances, appear to contribute to the underlying pathogenesis resulting in symptom generation, as well as neurodegeneration. Therapeutic strategies aimed at modulating axonal ion-channel dysfunction may prove therapeutically useful, particularly in ALS, with axonal excitability parameters utilized as primary or secondary biomarkers in future clinical trials.

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