Electrophysiological Abnormalities in SOD1 Transgenic Models in Amyotrophic Lateral Sclerosis: The Commonalities and Differences

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1. Introduction

Since its first description in 1874 by Charcot, the hallmark feature of ALS is the progressive degeneration of upper and lower motoneurons (Charcot, 1874). In the spinal cord, motoneuron degeneration starts long before symptom onset and advances in a size-related fashion, in which large-size alpha-motoneurons degenerate first followed by small-size alpha-motoneurons (Pun et al., 2006; Hegedus et al., 2007; Hegedus et al., 2008). There are conflicting reports regarding the survival of the smallest-sized spinal motoneurons, the gamma-motoneurons (Swash and Fox, 1974; Sobue et al., 1981). Despite its original description, the neuronal degeneration in ALS is not limited to motoneurons. Recent reports have shown evidence for degeneration of neurons in the brain (Karim et al., 1998; Lloyd et al., 2000; Maekawa et al., 2004) and interneurons in the spinal cord (Konno et al., 1986; Williams et al., 1990; Takahashi et al., 1993; Stephens et al., 2006).

Before their degeneration, spinal motoneurons experience progressive changes in their properties. These changes result from the pathological actions of the disease and the compensatory mechanisms of the nervous system to mitigate the neuronal malfunction. In this chapter, we describe the changes in the anatomical and electrical properties of spinal motoneurons in various genetic mouse models of ALS and critically analyze literature for the common and different pathological features across these models. We also present data from computer simulations showing the consequences of the alterations in properties of mutant motoneurons on cell excitability and dendritic processing of synaptic inputs. The presented computational analysis allowed for the identification of motoneuron alterations undetectable using standard electrophysiological methods. This information is essential for understanding motoneuron pathophysiology in ALS.

2. The changes in motoneuron anatomical properties

One of the earliest abnormalities in transgenic mouse models of ALS is the change in anatomy of spinal motoneurons. In the G85R model, it has been shown that mutant

motoneurons exhibit an increase in the overall cell size and branching pattern of dendrites (Amendola et al., 2007; Amendola and Durand, 2008). These changes appear in transgenic mice at 10 days after birth (P10), long before disease onset and life end-stage (225 and 240 days, respectively) (Bruijn et al., 1997). More specifically, in mutant motoneurons the total dendritic surface area and total dendritic length are increased by 58% and 65% relative to wild-type (WT) cells, respectively. For dendritic branching, the number of dendritic branches, terminal branches, branching nodes, and maximum branching order are increased by 93%, 89%, 97%, 37%, respectively (Fig. 1). On the other hand, the soma size (diameter and surface area) and primary dendrites properties (number, diameter, and cross-sectional area) are similar in WT and mutant motoneurons. Interestingly, the increase in number of branches occurs primarily in short branches (dendritic branches with path length < 100μm) and most of the anatomical changes take place over the middle dendrites (200μm - 500μm from the soma). Surprisingly, the longest dendritic length from the soma was not increased in mutant motoneurons, indicating that dendritic overbranching occurs mainly within the cell circumjacent. In other words, the cell does not swell into larger space in the cord but develops more branching within its dendritic spatial field.
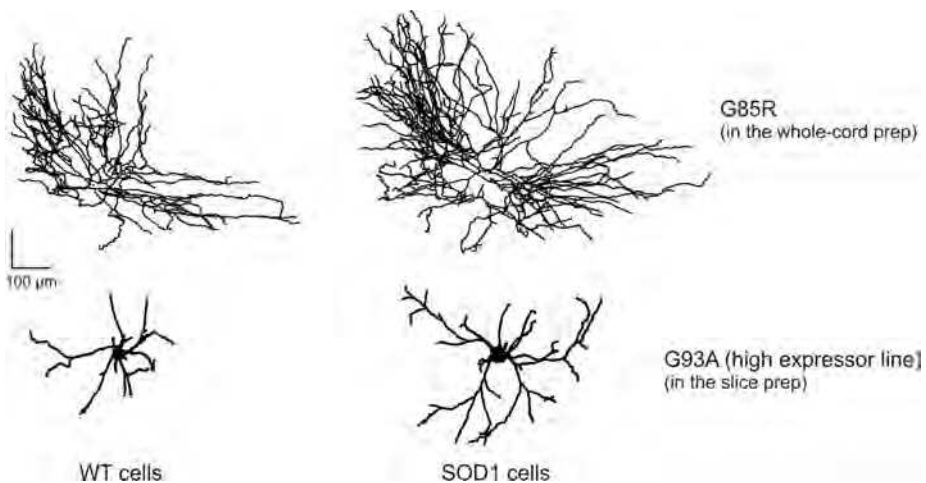

Fig. 1. Anatomical alterations in mutant motoneurons of the G85R (reconstructed from the whole-cord prep[1]) and G93A (high expressor line) (reconstructed from the slice prep.) transgenic models relative to WT cells. [1]: data from Amendola and Durand (2008).

Similar early abnormalities in the anatomical properties of motoneurons have been found in other transgenic mouse models of ALS. In the G93A (low expressor line), which expresses 8 copies of the human SOD1 gene, mutant motoneurons at P9 exhibit increased cell size, dendritic overbranching, and dendritic complexity (Filipchuk et al., 2010). The total dendritic length and number of branching nodes and terminal dendrites in mutant motoneurons were significantly increased relative to WT. Similar to the G85R transgenic model, these anatomical changes in the G93A (low expressor line) model occur early in the disease (at P9) way before symptom development (between 195 and 240 days) and end stage (between 240 and 270 days). Preliminary data from our laboratory obtained in the

slice preparation show that neonatal mutant motoneurons (P7) of the G93A (high expressor line), which expresses 25 copies of the human SOD1 gene, experience analogous alterations in their morphology and dendritic branching (Fig. 1), in which the soma surface area, numbers of dendritic branches and terminal dendrites increase significantly relative to WT.

Post-mortem morphological analysis of large neurons in the ventral horn of spinal cord indicated an increase in the initial segment diameter, by 36%, in ALS patients (Sasaki and Maruyama, 1992). Conversely, the soma area of these neurons was smaller by 16% relative to control (Sasaki and Maruyama, 1992).

The similar findings on the anatomical alterations of neonatal motoneurons in various transgenic models of ALS indicate that the increase in cell size and dendritic overbranching are characteristic abnormalities in motoneuron pathophysiology in ALS. However, it is unclear whether the alteration in motoneuron anatomy is a disease mechanism or an adaptive response (see section 7 for discussion). Furthermore, it is important to understand the functional ramifications of the motoneuron anatomical alterations on the cell excitability and electrical properties and to what extent the anatomical alterations contribute to motoneuron degeneration in ALS (see section 6).

3. The changes in motoneuron electrical properties

3.1 The change in input resistance

Changes in a number of motoneuron electrical properties were reported in the transgenic mouse models of ALS. However, one challenge in the field of ALS is the large variability in data of mutant motoneuron electrical properties, which makes recording from a large number of cells is important to allow statistical tests to detect significant differences in electrophysiological properties between WT and mutant motoneuron samples. Table 1 summarizes the changes in electrical properties of mutant motoneurons observed in the various ALS transgenic mouse models. The summary shows that the changes in electrical properties involve the motoneuron passive properties (e.g., cell input resistance), the size and shape of the action potential spike and afterhyperpolarization, the amplitude of voltage-sensitive ionic currents, and the motoneuron excitability and firing activity. However, some of these changes were inconsistent (i.e., not observed consistently) across the ALS transgenic models, whereas others were contradictory (i.e., opposite changes were observed). For instance, the decrease in input resistance of mutant motoneurons relative to WT is an inconsistent finding in ALS transgenic models (see table 1, column 7). This observation was seen in some studies (Bories et al., 2007; ElBasiouny et al., 2010; Quinlan et al., 2011), whereas other studies did not find a statistically significant difference between the input resistance of WT and mutant motoneurons (Pieri et al., 2003; Kuo et al., 2004; Kuo et al., 2005; van Zundert et al., 2008; Pambo-Pambo et al., 2009; Pieri et al., 2009; Filipchuk et al., 2010; Meehan et al., 2010). The changes in action potential size and width in mutant motoneurons, on the other hand, is a conflicting finding in which some studies reported an increase in spike height (van Zundert et al., 2008) and duration (Fuchs et al., 2008, 2009; Pambo-Pambo et al., 2009) relative to WT, whereas others reported reduction in spike height (Kuo et al., 2004; Pambo-Pambo et al., 2009; Filipchuk et al., 2010) and duration (Pieri et al., 2003; Quinlan et al., 2011).

Publication	Model	Preparation	Age	Neuron Type	$[Ca^{2+}]_o$	R_{in} in mutant neurons	V_{rest} and AP properties in mutant neurons	AHP in mutant neurons	PIC properties in mutant neurons	F-I gain & firing properties in mutant neurons
(Pieri et al., 2003)	G93A (high)	Embryonic Culture	E15: 7-12 days	SMNs	2mM	No diff	↓AP duration ↑AP repol rate	No diff	n/a	↑firing freq
(Kuo et al., 2004)	G93A (high)	Organotypic slice culture	P7: 3 weeks	SMNs	2.4mM	No diff	No diff	No diff	n/a	↑F-I gain ↑max firing
(Kuo et al., 2004)	G93A (high)	Embryonic culture	E13: 12-14 days	SMNs	2.8mM	No diff	↓AP height	No diff	n/a	↑F-I gain
(Kuo et al., 2005)	G93A (high)	Embryonic Culture	E13: 10-30 days	SMNs	2.8mM	No diff	Depolarized V_{rest} in high conductance MNs	No diff	↑NaPIC (measured in VC)	↓rheobase ↑F-I gain ↑spontaneous firing in high conductance MNs
(Bories et al., 2007)	G85R	Whole-cord	P6-10	SMNs	4mM	↓by 30%	No diff	No diff	n/a	↓F-I gain ↑rheobase
(van Zundert et al., 2008)	G93A (high)	Brainstem slice	P4-10	HGMNs & OINs	2mM	No diff	↑AP height	No diff	↑NaPIC (measured in VC)	↑firing freq
(Fuchs et al., 2008) (Fuchs et al., 2009)	G93A (high)	Brainstem slice	P80-120	HGMNs & OMNs	2mM	No diff	HGMNs (P120): ↑AP duration ↓AP repol rate	At P120: ↓$fAHP_{amp}$ ↑$mAHP_{amp}$	No diff	No diff
(Pambo-Pambo et al., 2009)	G93A (low)	Lumbar slice	P6-10	SMNs	2mM	No diff	↓AP height ↑AP half-width ↓AP rise & decay slopes Depolarized V_{rest}	No diff	No diff (measured in VC) ↓total PIC (estimated from ΔI data)	↑F-I gain (pulses)

Publication	Model	Preparation	Age	Neuron Type	$[Ca^{2+}]_o$	R_{in} in mutant neurons	V_{rest} and AP properties in mutant neurons	AHP mutal neuro
(Pambo-Pambo et al., 2009)	G85R	Lumbar slice	P6-10	SMNs	2mM	No diff	No diff	No d
(Pieri et al., 2009)	G93A (high)	Cell culture	E15: 11-12 days	CNs	2mM	No diff	↓AP threshold	n/a
(Meehan et al., 2010)	G127X	In-vivo lumbar cord	P240	SMNs	n/a	No diff	n/a	No d
(ElBasiouny et al., 2010)	G85R	Whole-cord	P8-10	SMNs	2mM	↓by 30%	No diff	No d
(Filipchuk et al., 2010)	G93A (low)	Whole-cord	P8-9	SMNs	2mM	No diff	↓ AP height ↓AP rise and decay slopes	No d
(Quinlan et al., 2011)	G93A (high)	Lumbar slice	P0-12	SMNs	2.5mM	↓by 19%	↓AP duration ↑AP rise and decay slopes	↓AH time constı

Table 1. Summary of the alterations in mutant motoneuron properties. SMNs: spinal motoneurons,
HGMNs: hypoglossal motoneurons, OMNs: ocular motoneurons, OINs: ocular interneurons. [Ca2+
concentration, R_{in}: input resistance, V_{rest}: resting membrane potential, AP: action potential, AHP: af
persistent inward current, F-I: frequency-current relationship. VC: voltage-clamp, ΔI: the difference
motoneuron recruitment and de-recruitment on a triangular current command, No diff: no differen

3.1.1 Effect of tissue preparation

The discrepancy in the changes of electrical properties of mutant motoneurons probably results partially from the differences in transgenic model types (e.g., G85R, G93A high or low expressor lines) and is compounded by the variability in experimental conditions such as animal age (e.g., neonatal, pre- or post-symptomatic adult motoneurons) and tissue preparation (e.g., cell culture, slice, whole-cord, or in-vivo), in addition to the recording conditions (e.g., extracellular Ca^{2+} concentrations) and measurement methods (current- or voltage-clamp). However, critical analysis of the changes in electrical properties of mutant motoneurons shows that common pathological features could be identified across the various ALS transgenic models, whereas other features could be related to experimental conditions. For instance, the decrease in input resistance was detected mainly in studies conducted on the whole-cord preparation (in which the brainstem and spinal cord are intact), but was not observed in studies on motoneurons in cell culture or slice preparations (Table 1). Because input resistance is an indirect measure of cell size, it becomes reduced in mutant motoneurons to reflect the enlargement in motoneuron anatomy discussed in the previous section. In the whole-cord preparation, the motoneuron dendrites are completely intact and their effect on input resistance is more easily detected. In the slice preparation, conversely, the motoneuron dendrites are truncated at the surface of the slice. The lack of the middle and distal portions of dendrites could mask the anatomical differences between WT and mutant motoneurons and would explain the disappearance of input resistance differences between WT and mutant motoneurons in the slice preparation. Table 2 shows a comparison of the input resistance values of neonatal motoneurons in the various transgenic models of ALS. In the G85R and G93A (low expressor line) models, the input resistance values in the whole-cord preparation (ElBasiouny et al., 2010; Filipchuk et al., 2010) were on average half of those in the slice preparation (Pambo-Pambo et al., 2009), indicating that nearly half of the dendrites were severed in the slice preparation. Strikingly, the decrease in input resistance of mutant motoneurons of the G93A (high expressor line) model was still detected in the slice preparation in Quinlan et al. (2011). This could indicate that the motoneuron anatomical alterations in the G93A (high expressor line) model are so extensive that they were still substantial in the slice preparation and/or the motoneuron membrane biophysical properties change considerably in the G93A (high expressor line) model and contribute significantly to the decrease in input resistance (see section 4). For cell culture, given that the trigger signal for the alteration in motoneuron anatomy is poorly understood and could result from the alteration in motoneuron excitability and synaptic inputs (Duch et al., 2008), mutant motoneurons in the cell culture preparation might not experience anatomical alterations due to their isolation (in the cell culture) from the pathological neural circuit (in the transgenic mouse spinal cord), leading to absence of differences in input resistance between WT and mutant motoneurons.

3.1.2 Effect of extracellular Ca^{2+} concentration

The measurement of input resistance values, and other electrical properties, in mutant motoneurons also depends on the extracellular concentration of Ca^{2+} ions in the recording solution. For instance, the input resistance value of neonatal motoneurons of the G85R model measured in 4mM of extracellular Ca^{2+} concentration (Bories et al., 2007) was double of that measured in 2mM of extracellular Ca^{2+} concentration (ElBasiouny et al., 2010) (see table 2). Although 2mM is typical, the variability in extracellular Ca^{2+} concentration (ranging

between 2mM and 4mM, see table 1) among the various ALS studies could contribute to the discrepancies in electrical properties of mutant motoneurons. This effect is produced because the level of extracellular Ca^{2+} concentration modulates the magnitude of the motoneuronal Ca^{2+}-gated (e.g., Cav 1.2, 1.3, and 2.2 channels) and Ca^{2+}-activated (e.g., SK and Ih channels) ionic currents, which regulate the motoneuron firing activity and affect the action potential and afterhyperpolarization properties.

Model	Publication	Preparation	$[Ca^{2+}]_o$	R_{in} WT/SOD1	Anatomical alterations
G93A (high)	Quinlan et al. (2011)	Slice	2mM	38.5/31.3 MΩ (↓ by 19%)*	Yes[1]
G85R	Bories et al. (2007)	Whole-cord	4mM	33.3/23.8 MΩ (↓ by 29%)*	Yes[2]
	ElBasiouny et al. (2010)	Whole-cord	2mM	16.2/11.4 MΩ (↓ by 30%)*	
	Pambo-Pambo et al. (2009)	Slice	2mM	34.5/33.1 MΩ ns	
G93A (low)	Pambo-Pambo et al. (2009)	Slice	2mM	34.5/37.2 MΩ ns	Yes[3]
	Filipchuk et al. (2010)	Whole-cord	2mM	18.5/16.1 MΩ ns	

Table 2. Comparison of the change in input resistance in mutant motoneurons (P6-P10) in the various ALS transgenic models. [1]: unpublished data, [2]: Amendola and Durand (2008), [3]: Filipchuk et al. (2010), *: statistically-significant ($p<0.05$), ns: not statistically-significant.

In sum, the reduction in input resistance of mutant motoneurons is a common, early pathological feature in the G93A (high expressor line) and G85R transgenic models, but not in the G93A (low expressor line). Also, this abnormality might sometimes not be evident depending on the tissue preparation used in the experiment.

3.2 The changes in action potential and afterhyperpolarization

A change in the action potential properties was frequently seen in mutant motoneurons relative to WT; however, these changes were conflicting across the transgenic models (Table 1, Fig. 2). In the G93A (high expressor line) model, neonatal spinal motoneurons frequently showed faster action potential rate of rise and decay and shorter action potential duration (Pieri et al., 2003; Quinlan et al., 2011), indicating an increase in transient and persistent Na^+ currents, which act to increase the excitability of mutant motoneurons. Signs of increased excitability were differently displayed in other studies as an increase in spike height (van Zundert et al., 2008), depolarization in resting membrane potential (Kuo et al., 2005), or reduction in action potential threshold (Pieri et al., 2009). Conversely, an increase in action potential duration and deceleration in rate of repolarization were observed in post-symptomatic adult hypoglossal motoneurons (Fuchs et al., 2009).
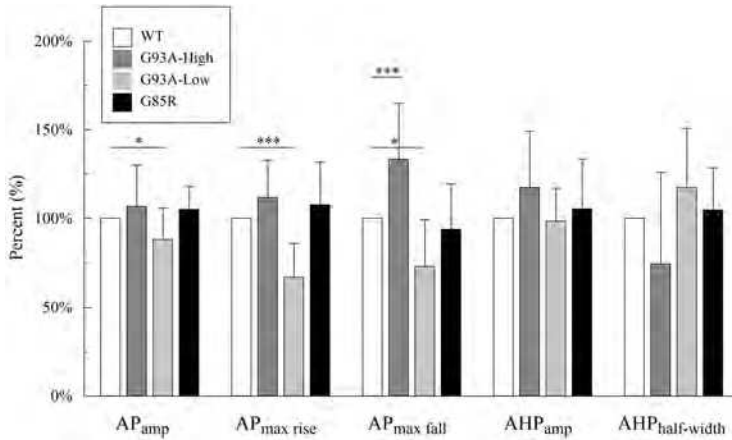
Fig. 2. The changes in action potential height (AP$_{amp}$), maximum rise (AP$_{max\ rise}$) and fall (AP$_{max\ fall}$) rates, and AHP amplitude (AHP$_{amp}$) and half-width (AHP$_{half-width}$) in mutant motoneurons of the G93A (high expressor line) [2nd bars][1], G93A (high expressor line) [3rd bars][2], and the G85R [last bars][2] transgenic models, relative to WT [1st bars]. All reported data from slice preparation. [1]: Quinlan et al. (2011), [2]: Pambo-Pambo et al. (2009).

In the G93A (low expressor line) model, neonatal spinal motoneurons exhibited a reduction in action potential height, increase in half-width, and deceleration in the rates of rise and decay (Pambo-Pambo et al., 2009; Filipchuk et al., 2010). In the G85R model, no changes were observed in the properties of the action potential regardless of the tissue preparation (whole-cord or slice) and extracellular Ca^{2+} concentration (2mM or 4mM) (Bories et al., 2007; Pambo-Pambo et al., 2009; ElBasiouny et al., 2010). Therefore, it appears that the alteration in action potential properties is not a common pathological characteristic, but depends on the ALS transgenic model type.

The after-spike afterhyperpolarization was not consistently altered in mutant motoneurons across the various transgenic models (Table 1, Fig. 2).

3.3 The change in persistent inward currents

Persistent inward currents are intrinsic Na$^+$ and Ca^{2+} currents that depolarize the membrane potential when activated. Contrary to transient ionic currents, these currents do not inactivate with prolonged membrane depolarization (Schwindt and Crill, 1977). A change in the motoneuron persistent inward current was frequently observed in mutant motoneurons (Table 1, Fig. 3); however, conflicting data were reported on the nature of this change (i.e., increase or decrease). In the G93A (high expressor line) model, an increase in the Na$^+$ persistent inward current of neonatal mutant motoneurons was consistently reported regardless of tissue preparation (cell culture and slice) or extracellular Ca^{2+} concentration (Table 1). In the G93A (low expressor line) and G85R models, persistent inward current measured in voltage-clamp protocols indicated no change in their amplitude between WT and mutant motoneurons, whereas persistent inward current estimated from the delta I (ΔI) technique indicated a reduction in their amplitude in mutant motoneurons relative to WT (Pambo-Pambo et al., 2009). The former is a more truthful result because voltage-clamp protocols allow direct

measurement of the persistent inward current, whereas the ΔI technique (which is obtained from the difference in injected current at motoneuron recruitment and de-recruitment on a triangular current command) provides an indirect estimate of the persistent inward current amplitude (Bennett et al., 2001) and is sensitive to the inactivation of transient Na^+ channels (Miles et al., 2005) and activation of residual outward currents present in the motoneuron membrane (Hamm et al., 2010). However, it should be noted that the number of cells in which the voltage-clamp measurements were obtained was small. In the infrequently studied G127X transgenic model, an increase in the persistent inward current amplitude was estimated from the motoneuron firing profile (Meehan et al., 2010). However, this result is unconfirmed given that firing profile in mutant motoneurons would be also influenced by any differences in inward or outward currents and electrical properties of mutant motoneurons. Accordingly, the change in amplitude of persistent inward currents is not a common abnormality in the various ALS transgenic models. However, it appears that persistent inward currents are increased only in the G93A (high expressor line) transgenic model.
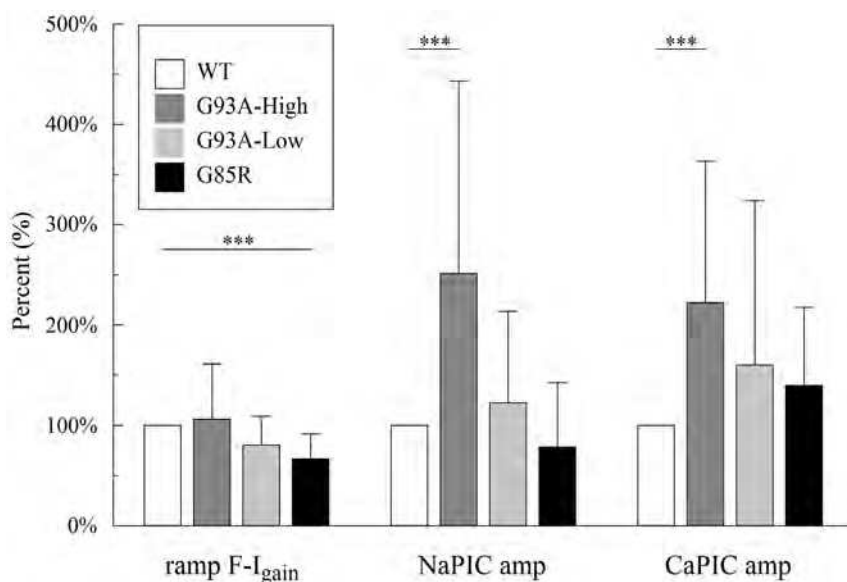

Fig. 3. The changes in ramp frequency-current relationship gain (ramp F-I_{gain}), sodium (NaPIC) and calcium (CaPIC) persistent inward currents in mutant motoneurons of the G93A (high expressor line) [2nd bars][1], G93A (high expressor line) [3rd bars][2], and the G85R [last bars][2] transgenic models, relative to WT [1st bars]. All reported data from slice preparation. [1]: Quinlan et al. (2011), [2]: Pambo-Pambo et al. (2009).

3.4 The change in motoneuron gain and firing activity

A change in the motoneuron gain (i.e., F-I relationship slope) or firing activity was commonly seen in mutant motoneurons, but conflicting observations were also reported. In the G93A (high expressor line) transgenic model, the firing activity and gain of mutant spinal motoneurons were consistently increased in cell culture (Pieri et al., 2003; Kuo et al.,

2004; Kuo et al., 2005; Zona et al., 2006; Pieri et al., 2009), but not in the slice (Quinlan et al., 2011) (Table 1). In the G85R transgenic model, the gain of mutant motoneurons was consistently reduced relative to WT (Bories et al., 2007; Pambo-Pambo et al., 2009). In the G93A (low expressor line) transgenic model, the gain of mutant motoneurons was increased in the slice preparation (Pambo-Pambo et al., 2009), but did not change in the whole-cord preparation (Filipchuk et al., 2010). The disagreement in reports on the change in motoneuron gain in the various transgenic models could be partially due to the variation in tissue preparation and extracellular Ca^{2+} concentration. Motoneuron dendrites have active conductances, which influence the motoneuron gain. Therefore, the portion of the motoneuron dendrites available during recording in the slice preparation would indirectly affect the measurement of the motoneuron gain in WT and mutant motoneurons. Also, the effect of extracellular Ca^{2+} concentration on the amplitude of Ca^{2+}–activated and Ca^{2+}–gated currents would influence the motoneuron gain measurement based on the degree of existence of these channels in WT and mutant motoneurons. Taken collectively, the change in gain and firing activity of mutant motoneurons is inconsistent within and across the various ALS transgenic models.

3.5 Motoneuron excitability in the various ALS transgenic models

Despite the discrepancy in data on the excitability of mutant motoneurons in the various ALS transgenic models, some insights could be attained from the trend in electrical properties change (Table 3). In the G93A (high expressor line) model, most changes in electrical properties push toward increased excitability of mutant motoneurons. For instance, the increases in action potential height and rise and decay rates and the reduction in action potential width are signs of elevated transient and persistent Na^+ currents that enhance the motoneuron excitability of mutant motoneurons. Also, the increase in persistent inward current amplitude further acts to enhance the motoneuron excitability. Collectively, these mechanisms push toward increased excitability of mutant motoneurons (i.e., excitability enhancement mechanisms). On the other hand, the increase in motoneuron size and reduction in input resistance are mechanisms that counteract increased excitability (i.e., excitability suppressive mechanisms) because the motoneuron becomes harder to recruit. It is unclear whether the excitability enhancement or suppressive mechanisms are disease or compensatory processes; however, the net effect of these mechanisms is increased excitability (i.e., hyperexcitability) of mutant motoneurons in the G93A (high expressor line) model (Table 3). This supposition is supported by the numerous reports on increased gain, although from cultured motoneurons, of mutant motoneurons in this transgenic model.

In the G93A (low expressor line) model, all changes in electrical properties indicate a reduction in the excitability of mutant motoneurons (i.e., hypoexcitability) (Table 3). These changes involve increase in motoneuron size, reduction in input resistance, smaller and broader action potential spikes, and slower rise rate (Table 3). The last two observations are indicative of a decrease in transient and persistent Na^+ currents in mutant motoneurons relative to WT. In contrast to these data, the gain of mutant motoneurons measured using long pulses was higher than that of WT motoneurons; however, the gain measured using current ramp was not different from WT motoneurons (Pambo-Pambo et al., 2009). Taken collectively, more evidence is available on reduced excitability of mutant motoneurons in the G93A (low expressor line).

Model	MN size	R_{in}	AP/AHP	PIC	Gain	Excitability
G93A (high)	↑↑	↓↓	Higher & briefer spikes Faster rise & decay rates	↑↑	↑↑/--	Hyperexcitability
G93A (low)	↑↑	--	Smaller & broader spikes Slower rise & decay rates	--	↑↑/--	More evidence on hypoexcitability
G85R	↑↑	↓↓	--	--	↓↓	Hypoexcitability

Table 3. Summary of changes in motoneuron properties in the various ALS transgenic models. MN: motoneuron, Rin: input resistance, AP/AHP: action potential and afterhyperpolarization, ↑↑: increase, ↓↓: decrease, --: no change.

In the G85R transgenic model, not many changes were reported in the electrical properties of mutant motoneurons; however, these changes were consistent with reduced excitability and are supported by the decrease in gain of mutant motoneurons (Table 3). In conclusion, motoneuron pathophysiology is different in the various transgenic mouse models of ALS and their excitability varies from hypo- to hyperexcitability. This disparity could be related to the number of copies of the human SOD1 gene or the severity of the disease (transgenic models with high copy number of SOD1 genes have more aggressive ALS than transgenic models with low copy number of SOD1 genes). Transgenic models with a low number of SOD1 copies (e.g., G85R and G93A-low expressor line) showed a tendency for reduced excitability of mutant motoneurons, whereas transgenic models with a high number of SOD1 copies (e.g., G93A-high expressor line) showed a tendency for increased excitability of mutant motoneurons. These differences should be considered when studying the motoneuron pathophysiology in these ALS transgenic models.

4. Effect of anatomical alterations on motoneuron electrical properties

The enlarged anatomy of mutant motoneurons has consequences for their electrical properties and firing behaviour. Computer simulations have been used to assess the effect of enlargement in motoneuron anatomy and its contribution to the changes in electrical properties (ElBasiouny et al., 2010). Realistic computer models were developed from the reconstructed morphologies of WT and mutant motoneurons of the G85R model and were optimized to replicate the electrophysiological recordings obtained from individual cells. For the 30% reduction in input resistance of mutant motoneurons relative to WT, computer simulations showed that one third of this reduction (i.e., input resistance decrease by 10%) is due to the enlargement of motoneuron anatomy, whereas two thirds of this reduction (i.e., input resistance decrease by additional 20%) is due to a decrease in the specific membrane resistance of the motoneuron membrane. The specific membrane resistance represents the number of leak channels available in a patch of cell membrane and its decrease means that there are more ion channels inserted into the membrane (i.e., the cell has higher conductance). Comparison of WT and mutant motoneuron models

indicated a 25% decrease in the somatic and dendritic specific membrane resistance of the mutant models. Therefore, the simulation results indicate that the enlargement in motoneuron anatomy does not fully account for the reduction in input resistance as previously suggested by Bories et al. (2007). This is because the additional dendritic branches causing the increased surface area are electrotonically distant from the soma, the site of input resistance measurement. This electrotonic separation reduces the influence of the additional dendritic area at the soma. Thus, a 60% increase in total surface area of mutant motoneurons causes only a 10% decrease in cell input resistance (not 40% as would be predicted from the reciprocal of surface area increase of an electrotonically compact sphere). Secondly, the simulation results indicate that the reduction in input resistance of mutant motoneurons is a function of two factors: the enlargement in motoneuron anatomy and the decrease in specific membrane resistance; the weights of these factors determine the magnitude of input resistance reduction. This could explain why the enlargement of motoneuron anatomy was not always associated with reduction in input resistance as in the case of the G93A (low expressor line) model (see Table 2), suggesting an insignificant change in the membrane specific membrane resistance in this transgenic model. In contrast, the significant reduction in input resistance of mutant motoneurons of the G93A (high expressor line) model despite the truncation in motoneuron dendrites in the slice preparation in Quinlan et al. (2011) might indicate a substantial change in the specific membrane resistance in that transgenic model.

The effects of enlargement in mutant motoneuron anatomy are not only limited to cell input resistance, but also extend to cell firing properties. For instance, the enlargement in mutant motoneuron anatomy would be expected to cause reductions in the motoneuron F-I gain and initial and maximum firing rates and increases in rheobase current and total cell capacitance. Given that no change was seen in some of these properties of mutant motoneurons indicate that compensatory mechanisms have masked those effects (e.g., upregulation in motoneuron transient and persistent inward currents). Estimating the contribution of anatomy enlargement to the changes in mutant motoneurons firing activity and identifying potential ionic mechanisms for masking these changes using computer simulations would be an important step in revealing hidden alterations in motoneuron properties and improving our understanding of ALS pathogenesis.

5. The changes in synaptic inputs to motoneurons

In ALS patients and transgenic models, synaptic inputs converging onto motoneurons experience changes during disease progression. These changes involve loss of specific types of synaptic inputs, rearrangement of the synaptic contacts, and alteration in the size of synaptic boutons. For instance, it has been shown that cholinergic synapses on lumbar motoneurons are lost in ALS patients (Nagao et al., 1998). This loss starts before motoneuron degeneration. Given that cholinergic synapses provide inhibitory input to motoneurons (Nagy et al., 1993), their loss could alter the balance toward more excitatory inputs, leading to motoneurons overactivation. This prediction has been confirmed in G93A (high expressor line) transgenic mice in which the ratio of inhibitory to excitatory synapses was reduced due to the loss of inhibitory boutons (loss was mediated by nitric oxide) and increase in excitatory boutons (Sunico et al., 2011). Furthermore, residual inhibitory boutons exhibit shorter active zone length and smaller synaptic vesicle density (Sunico et al., 2011). Along the same line, there was a progressive increase in the

excitability of interneurons, through NMDA receptors, that starts in the presymptomatic stage of ALS (Jiang et al., 2009).

Computer simulations of mutant motoneuron models predicted a reduction in the efficacy of dendritic processing of synaptic inputs early in ALS (ElBasiouny et al., 2010). The amplitude of somatic excitatory postsynaptic potentials (EPSPs) was reduced in mutant motoneurons by 15%, relative to WT. This reduction was comparable to experimental measurements of EPSP amplitudes in the G85R transgenic model (Bories et al., 2007). The computer simulations showed that the reduction in EPSP amplitude resulted from the alteration in various motoneuron properties such as: the increase in motoneuron morphology, the decrease in specific membrane resistance, and the increase in dendritic active conductance activation. The changes in these properties counteract each other and produce the net reduction in EPSP amplitude. These changes were consistent in synaptic inputs with different dynamics (i.e., slow and fast inputs) (ElBasiouny et al., 2010).

6. The functional ramifications of the alteration in motoneuron properties

The combined changes in motoneuron properties (anatomical, electrical, biophysical) and synaptic inputs converging on them in ALS alter the input-output function of mutant motoneurons and affect their recruitment. For instance, the reduction in input resistance of mutant motoneurons makes them harder to recruit. Similarly, the enlargement in motoneuron anatomy and development of dendritic overbranching increase the attenuation of excitatory and inhibitory post synaptic potentials as they flow to the soma along the dendrites and reduce their efficacy. Computer simulations of reconstructed morphologies of WT and mutant motoneurons indicated a reduction in the efficacy of slow and fast synaptic inputs (by ≈20%) in mutant motoneurons (ElBasiouny et al., 2010). Half of this efficacy reduction was due to the dendritic overbranching, whereas the second half was due to the decrease in specific membrane resistance, leading to increased signal loss through leak conductances. The simulations demonstrated that reduction in synaptic efficacy was still present despite the upregulation in the dendritic active conductances mediating persistent inward current. These combined reductions in cell input resistance and synaptic input efficacy push toward reduced excitability of the cell. It is unclear whether these changes represent disease or protective mechanisms (see next section for discussion).

7. Anatomical alterations versus excitability changes: Disease versus neuroprotective mechanisms?

The individual changes seen in properties of mutant motoneurons could be a disease mechanism, which produces a physiological malfunction, or a neuroprotective (i.e., compensatory) mechanism of the nervous system, which mitigates the physiological malfunction caused by the disease. These mechanisms develop sequentially and act antagonistically. Given that ALS pathogenesis is still poorly understood (i.e., disease mechanisms are not identified yet), it is unfeasible to categorize the changes in mutant motoneurons to disease or compensatory mechanisms with assertion; however, some hypotheses could be formulated from available data regarding the nature of these changes. For instance, the relationship between the alterations in anatomy and excitability of mutant motoneurons is paradoxical. In the G93A (high expressor line) model, the enlargement in motoneuron anatomy and concomitant increase in persistent inward current have opposite

actions on the excitability of motoneurons. Two hypotheses are described to infer the nature of these mechanisms. The first hypothesis is based on the effect of endurance training on healthy and transgenic mice. In healthy mice, endurance running exercise reduced the excitability of healthy motoneurons and increased their size, as suggested from their input resistance and cell capacitance measurements (Beaumont and Gardiner, 2002, 2003). In G93A mice, endurance running exercise reduced motor performance and shortened the life span of transgenic mice (Mahoney et al., 2004). Assuming that detrimental effects on transgenic mice would result from approaches that promote disease mechanisms, it is therefore plausible to suggest that anatomy enlargement of motoneurons is potentially an ALS disease mechanism. Consequently, the increase in motoneuron persistent inward current is potentially a compensatory mechanism to enhance the motoneuron excitability and offset the effect of enlarged anatomy. In this scenario, the additional dendrites would not possess active conductances and would reduce the motoneuron excitability by reducing the cell input resistance. However, this hypothesis is challenged by the beneficial effect of riluzole (the only FDA-approved drug available for ALS patients), which reduces motoneuron excitability and extends the life span of ALS patients (Bensimon et al., 1994; Miller et al., 1996) and transgenic mice (Gurney et al., 1998), suggesting increased excitability as the disease mechanism.

The second hypothesis is based on the relationship between the intrinsic motoneuron excitability and dendrite anatomy. When potassium channels were genetically manipulated to increase or decrease the motoneuron excitability, the overall motoneuron size was increased in both conditions with increased dendritic branch formation in the former case or dendritic branch elongation in the latter (Duch et al., 2008). Given that the anatomical alterations seen in mutant motoneurons resemble some features of those produced in response to increased excitability, this suggests that anatomy enlargement of mutant motoneurons is potentially a compensatory mechanism, whereas the increase in motoneuron excitability is potentially the disease mechanism. In this scenario, the pathologically-formed dendrites could contribute to the disease-state by having dendritic active conductances, which would dramatically increase the magnitude of persistent inward currents and enhance the motoneuronal excitability. The reduction in input resistance of mutant motoneurons would result from the increase in cell size. This hypothesis is supported by the beneficial effect of riluzole on mutant motoneurons survival by suppressing their excitability as explained above. Because motoneuron hyperexcitability is not a common feature of ALS transgenic models (hyperexcitability appears in models with high copy number of SOD1 gene, Table 3), riluzole's effect could be more pronounced in the more aggressive models of ALS that exhibit hyperexcitability, but less effective in the mild models of ALS (with less copy number of SOD1 gene) that exhibit hypoexcitability. This prediction might explain the discrepancy in studies on riluzole's efficacy in the various ALS transgenic models (for review see Bellingham, 2011). More data are needed to divulge the nature of these mechanisms.

In the G85R and G93A (low expressor line) models, alterations in anatomy and excitability of mutant motoneurons are observed, with no change in persistent inward current amplitude (Table 3). This disparity in the relationship between persistent inward current amplitude and motoneuron excitability in the various ALS transgenic models could be due to the difference in pace of disease progression in these models. In other words, disease and compensatory mechanisms advance at faster rates in aggressive ALS models (with high

copy number of SOD1 genes) than in mild ALS models (with low copy number of SOD1 genes). This supposition is supported by the earlier disease onset and shorter life span in the G93A (high expressor line) than in the G85R and G93A (low expressor line) models (Turner and Talbot, 2008). Longitudinal studies in in-vivo mouse preparations in which the changes in motoneuron excitability, persistent inward currents, and anatomical properties in the various ALS transgenic models could be monitored at short time intervals during disease progression are needed to examine the development of these mechanisms.

8. Conclusion

Numerous alterations in the anatomical and electrical properties of mutant spinal motoneurons take place in the first two postnatal weeks, long before disease onset. Many of these alterations are inconsistent and sometimes contradictory; however, critical analysis of these alterations allowed for the identification of common pathological features within and across the various transgenic models of ALS. The enlargement in anatomy and reduction in input resistance of mutant motoneurons are characteristic features in the various transgenic models of ALS, whereas the alterations in motoneuron excitability and ionic currents (both transient and persistent) differ across transgenic models. To date, it is unfeasible to identify which of these alterations is an action of the disease (i.e., disease mechanism) or a reaction of the nervous system (i.e., compensatory mechanism) and more experiments are needed to elucidate the nature of these alterations. Computer simulations of realistic models of WT and mutant motoneurons allowed for the identification of hidden alteration in the biophysical properties of mutant motoneurons and demonstrated that synaptic efficacy is reduced in mutant motoneurons. It would be important to have extensive computational analysis of motoneuron properties in the various ALS transgenic models at various points in time during disease progression to identify and monitor the immeasurable changes in membrane properties of mutant motoneurons. This information would be expected to improve significantly our understanding of motoneuron pathophysiology in ALS.
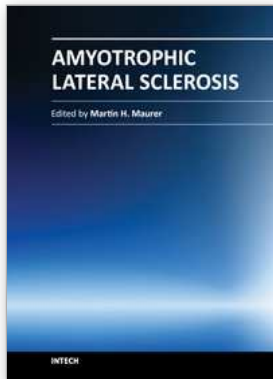
9. Acknowledgment

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Though considerable amount of research, both pre-clinical and clinical, has been conducted during recent years, Amyotrophic Lateral Sclerosis (ALS) remains one of the mysterious diseases of the 21st century. Great efforts have been made to develop pathophysiological models and to clarify the underlying pathology, and with novel instruments in genetics and transgenic techniques, the aim for finding a durable cure comes into scope. On the other hand, most pharmacological trials failed to show a benefit for ALS patients. In this book, the reader will find a compilation of state-of-the-art reviews about the etiology, epidemiology, and pathophysiology of ALS, the molecular basis of disease progression and clinical manifestations, the genetics familial ALS, as well as novel diagnostic criteria in the field of electrophysiology. An overview over all relevant pharmacological trials in ALS patients is also included, while the book concludes with a discussion on current advances and future trends in ALS research.

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