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14. ABSTRACT
The long-term objective of this exploratory research project was to uncover cellular mechanisms involved in adaptation in the excitability of spinal motoneurons, in response to changes in overall motor activity. The central hypothesis of the work was that alteration of the function of KCNQ/Kv7.2 potassium channels and changes in the properties of the axonal initial segment (AIS) are the primary mechanisms by which motoneurons adapt to prolonged network activation (the cellular equivalent of physical fatigue). Work to achieve the goals of this proposal included a combination of immunohistochemistry and electrophysiological techniques to investigate the

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Report Title

Final Report: Section 8.4 Neurophysiology and Cognitive Neuroscience: Adaptive modulation of excitability in motor neurons

ABSTRACT

The long-term objective of this exploratory research project was to uncover cellular mechanisms involved in adaptation in the excitability of spinal motoneurons, in response to changes in overall motor activity. The central hypothesis of the work was that alteration of the function of KCNQ/Kv7.2 potassium channels and changes in the properties of the axonal initial segment (AIS) are the primary mechanisms by which motoneurons adapt to prolonged network activation (the cellular equivalent of physical fatigue). Work to achieve the goals of this proposal included a combination of immunohistochemistry and electrophysiological techniques to investigate the extent that changes in the somatodendritic and AIS responsiveness of motoneurons and the shape of the AIS can explain the complex effects of prolonged spinal network activation on motoneuron excitability. Specific KC Q/Kv7 potassium channel modulating drugs will be used to dissect the contribution of the channels to the excitability of spinal motoneurons in both baseline and adapted states, differentiating their somatodendritic and axonal contributions. Immunohistochemical labeling of the AIS in baseline and adapted neurons can identify activity-dependent changes in AlS geometry and distance from the soma. We also developed detailed computational models of spinal motoneuron activity before and after persistent network activation.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
02/14/2017	1	Joseph Lombardo, Melissa A. Harrington. Nonreciprocal mechanisms in up- and downregulation of spinal motoneuron excitability by modulators of KCNQ/K, Journal of Neurophysiology, ( ): 2114. doi:
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(c) Presentations

Lombardo, J. and M.A. Harrington, 28.08 / AAA10 - Non-reciprocal effects of KCNQ/Kv7 channel modulation on the excitability of spinal motoneurons in mouse neonates. Society for Neuroscience Annual Meeting
November 2016, San Diego, CA.

Tharaneetharan, A. and M.A. Harrington, 523.17 / PP6 - Multielectrode array studies with culture models of motor neurons. Society for Neuroscience Annual Meeting, November 2016, San Diego, CA.

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Received Book

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TOTAL:

Patents Submitted

Patents Awarded

Awards

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FTE Equivalent:	
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Names of Post Doctorates

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Names of Faculty Supported

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Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):.....	0.00
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Names of Personnel receiving masters degrees

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Names of personnel receiving PHDs

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Scientific Progress

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Final Progress report

Forward

Spinal motoneurons comprise the final common pathway of voluntary nervous system output, transforming the results of the dynamic interaction of sensory inputs (via proprioception as well as external stimuli) and volitional impulses into motor acts. Understanding the neuromuscular transformation - how motoneurons integrate information and modulate their output to perform motor task under a variety of changing conditions, is a fundamental challenge in neuroscience with implications for rehabilitation, advanced prosthetics, brain-machine interfaces, humanoid robotics, and other biologically-inspired systems.

Motoneurons have long been thought to function simply as relays to muscle activation, however a growing body of work demonstrates that activity-dependent plasticity occurs in spinal motor neurons during development, as well as later in life with skills acquisition and maintenance, and in response to trauma and disease [1]. While synaptic plasticity is the most studied mechanism of learning [3], recent work has demonstrated that there is plasticity in the intrinsic electrical properties of neurons, and this type of plasticity is involved in motor learning. Understanding the mechanisms by which the output of spinal motor can be modified by both increased and decreased activity is crucial to understanding how motor commands are transformed into muscle contraction.

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Background & Problem Statement

A growing body of work is demonstrating the plastic potential of motoneurons. In primates and rodents, operant conditioning of the spinal stretch reflex over the course of weeks changes the axonal conduction velocity and excitability of motoneurons [4,5]. Similarly in rats, 16 – 20 weeks of either exercise training or inactivity changes the electrical properties of motor neurons in opposite directions [6-8]. "Exercised motoneurons" have a hyperpolarized threshold voltage (resulting in greater responsiveness to activating stimuli) as well as faster spike rise times, and a lower spike frequency adaptation (SFA) [7,8]. SFA is a decline in firing frequency with constant stimulation [9-11] that is thought to be important for matching motoneuron firing rate to the requirements of the motor task and the contractile properties of the muscle [12-15].

Another important activity-dependent modulation of neuromotor system function is central fatigue, in which changes in the activity of supraspinal and spinal motor centers limit effective muscle contraction well before the point at which muscles fail [16]. The activity-related adaptation of motoneurons' excitabity after prolonged network activation that we observe may be related to central fatigue. Spike Frequency Adaptation has been implicated as a contributor to central fatigue [10,13,16], while other work has implicated neuromodulatory input both in central fatigue and as a modulator of SFA [17]. While potentially related to SFA, the adaptive change in excitability that we observe in motoneurons emerges and persists over a different time course than SFA and appears to involve different mechanisms.

Recent work has demonstrated that the axonal initial segment (AIS) is not merely a trigger zone for action potentials, but rather is a site for complex neuronal processing. In cortical neurons, the recent activity of a neuron has been shown to change ion channel expression at the AIS, as well as altering its size or its distance from the soma [18-20]. For example, in dissociated hippocampal cultures as well as acute hippocampal slices, prolonged activation with high extracellular K^+ moves the AIS farther from the soma of excitatory neurons, and the movement reverses when neurons are returned to non-depolarized conditions [21,22]. Such modifications have a strong adaptive significance because the position and geometry of the action potential initiation zone strongly influences the ability of a neuron to recognize specific spiking patterns, and thus to filter or encode specific network information [22,23].

The long-term objective of this exploratory research project was to uncover cellular mechanisms involved in adaptation in the excitability of spinal motoneurons, in response to changes in overall motor activity. The central hypothesis of the work was that alteration of the function of KCNQ/Kv7.2 potassium channels and changes in the properties of the axonal initial segment (AIS) are the primary mechanisms by which motoneurons adapt to prolonged network activation (the cellular equivalent of physical fatigue).

Major Results

We have used acute slices of the lumbar spinal cord from neonatal mice (post-natal days 3 – 8) to investigate the effect of persistent activation of spinal motor networks. Multielectrode array (MEA) recordings with the MED64 (a 64 electrode planar array) show that exposure to high (10 mM) K+ over 30 minutes increases neural activity that is sustained until K+ concentrations are returned to normal (Fig. 1).

High K^+ treatment has been shown to generate rhythmic patterns of activity in spinal cord slices that resembles the activity occurring in locomotion [24], so prolonged incubation in high K^+ is likely to mimic the effects of sustained motor activity. In patch clamp studies of motoneurons recorded in acute spinal cord slices from neonatal mice we have observed that sustained (≥ 30 min) network activation with high (10 mM) K^+ leads to an adaptive (homeostatic) decrease in intrinsic excitability. After sustained activation we observe a hyperpolarization of the resting membrane potential, an increase in the current threshold, and a decrease in input resistance (Fig. 2; next page) all changes associated with decreased excitability.

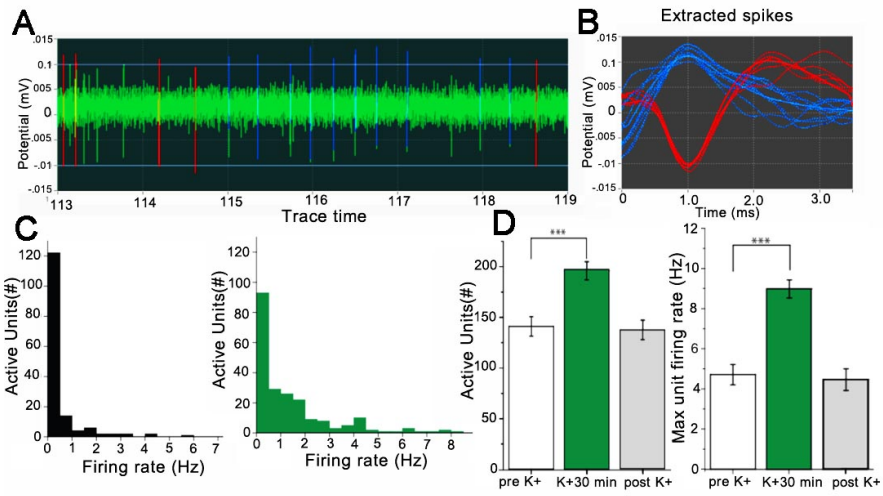

Figure 1. High K+ activates spinal cord neurons in acute slices: Results from multi-electrode recording from ventral horn slices before and after exposure to 10 mM K+. **A, B.** Spike sorting was used to track changes in neural activity. **A.** Representative trace with spikes of multiple units identified (red & blue) and **B.** Superimposed spike profiles for the two units in the recording. **C.** Representative histograms showing the number of active units in different frequency classes before (black) and after (green) 30 min in high K+. **D.** Pooled data of changes in the number of active units and maximal firing frequency before (white) and after 30 minutes in high K+ (green) and after washout (gray).

Separating the threshold of the AIS from that of the somatodendritic region highlighted an interesting difference in the change in excitability after prolonged network activation. Taking the first time derivative (dV/dt) of the voltage recordings of action potentials allows temporal separation of spike initiation in the AIS and somato-dendritic region. The first inflection point in the plot reflects invasion of the soma by the AIS spike, while the second inflection is thought to coincide with the onset of the somato-dendritic spike [25,26]. Our results data show that high K+ treatment depolarizes the voltage threshold for the AIS while hyperpolarizing the threshold for the AIS (Fig. 3, next page).

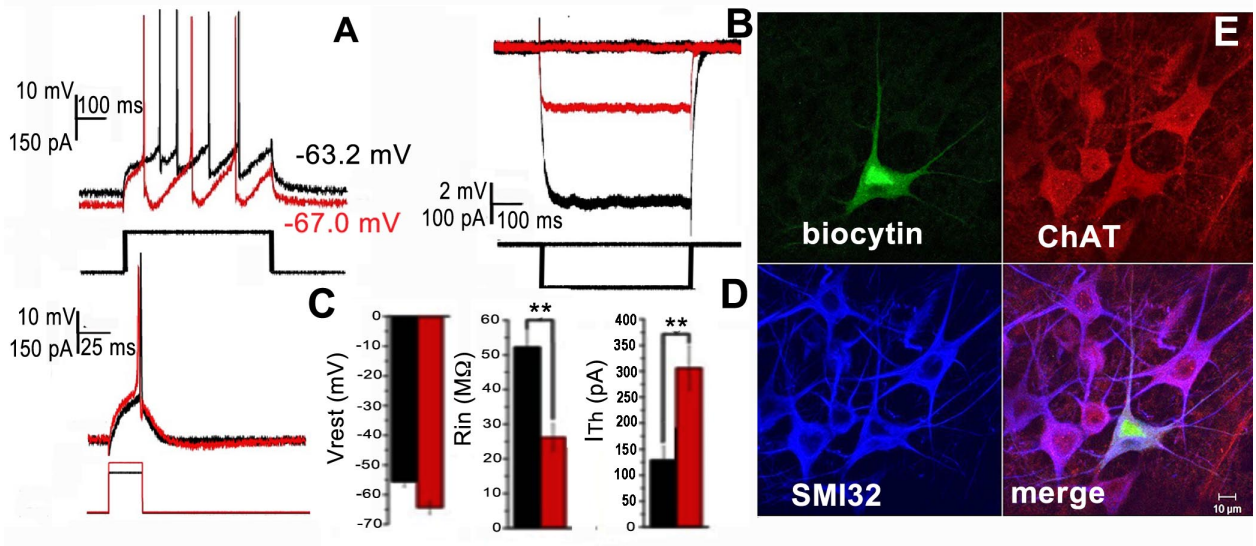

Figure 2. High K⁺ treatment decreases intrinsic excitability in motoneurons. **A**: Representative responses of motoneurons in untreated (black) and incubated (red) spinal cord slices for at least 30 min in high (10 mM) K⁺ 500 ms current injection of 300 pA at their resting membrane potentials. **B.** 500 ms hyperpolarizing current injections (-200 pA) at -70 mV common membrane potential. **C.** Minimal supra-threshold 25 ms current pulse (Δ10pA) at a common membrane potential of – 70 mV **D.** Mean ± SE of intrinsic excitability properties (Vrest, resting membrane potential; Rin, input resistance; and ITh, threshold current; Mann-Whitney U test: **, $p < 0.01$. Untreated, n = 11; treated, n = 12. **E**. IHC labeling with motoneuron-specific markers of a neuron filled with Alexa Fluor 488-Biocytin during recording.

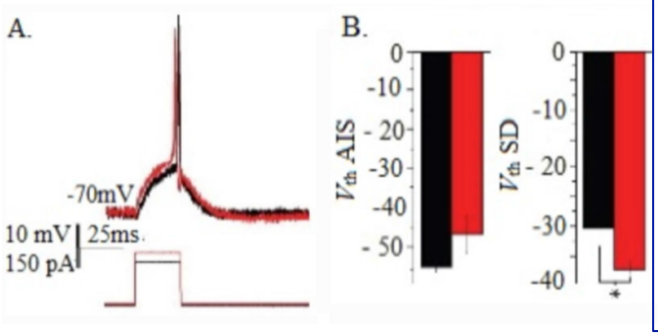

Figure 3. High K+ treatment differentially affects somatodendritic and AIS excitability in motoneurons. **A.** Minimal supra-threshold 25 ms current pulse (Δ10pA) responses at a common membrane potential of – 70 mV (intensities: 700 pA for naïve, 800 pA for incubated) of motoneurons in naïve (black) and spinal cord slices incubated for 30 min. in high 10 mM K+(red). **B.** Mean ± SE of voltage threshold (Vth) for the somatodendritic region (Vth SD) and the AIS (Vth AIS) spike voltage thresholds of motoneurons in naïve (black) and high K+ incubated (red) spinal cord slices). * t test, $p < 0.05$, n = 5.

These apparently contradictory results – a hyperpolarized spike threshold (increased excitability) in the somato-dendritic region combined with depolarization of the spike threshold (decreased excitability) in the AIS are similar to what has been observed in other systems with inhibitory channels that are activated differently in different cellular compartments [27]. The changes in the electrical properties of motoneurons we observe after sustained network activation in particular the hyperpolarized resting membrane potential, reduced input resistance, and increased threshold current, are consistent with alterations in KCNQ/Kv7 channels that underlie the M current, and changes in KCNQ/Kv7 channels have been observed to play a role in alterations of the intrinsic excitability properties of other types of neurons in a manner similar to what we observe in motoneurons. For example, inhibition of KCNQ/K_v7 channels has been shown to hyperpolarize spike threshold in the soma of hippocampal neurons [28,29], while axonal recordings in cortical pyramidal neurons show that inhibition of KCNQ/K_v7 channels depolarizes the AIS spike threshold without changing the somato-dendritic spike threshold [30].

Our own results strongly implicate KCNQ/Kv7 channels in the main intrinsic excitability changes (resting membrane potential, input resistance and current threshold) that we observe. KCNQ/Kv7 channels are voltage–gated K+ channels that activate at sub-threshold potentials with a slow time-course. These channels do not inactivate, thus generating a steady outward current that assists in stabilizing the membrane potential in the presence of depolarizing currents [31]. Our results show that after the application of the specific KCNQ/Kv7 blocker, XE991, the main parameters of spinal motoneuron intrinsic excitability are not significantly different for acute slices treated for 30 minutes with high (10 mM) K^+ and in untreated slices. This suggests that spinal motoneuron KCNQ/Kv7 channels are the main target of the modulation induced by prolong spinal network activation (Fig. 4).

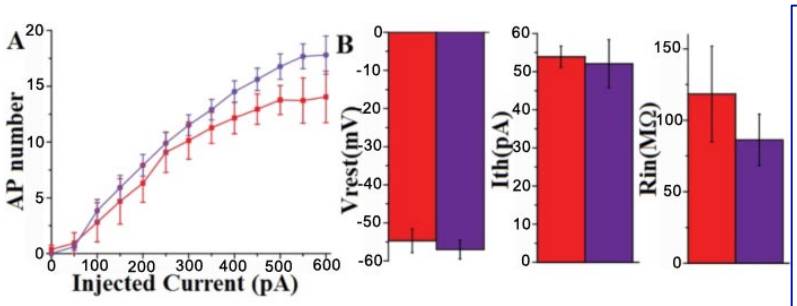

Figure 4. XE991 abolishes the reduction in intrinsic excitability of motoneurons. **A**: Effect of XE991 (3 μM) on action potential input-output curves of treated (purple) and untreated (red) slices in high (10 mM) K^+ for at least 30 min. **B.** Mean ± SE of intrinsic excitability properties (Vrest, resting membrane potential; Rin, input resistance; and threshold current; (Ith) of the same neurons - treated: N = 8, untreated: N = 4).

To generate hypotheses about how to resolve the apparent contradiction in changes in somato-dendritic and AIS excitability after persistent activation (hyperpolarizing the voltage threshold of the somato-dendritic region suggesting increased excitability, while depolarizing the threshold at the AIS, suggesting decreased excitability; see Fig. 3) and to understand what the consequences might be for overall neuronal responsiveness of the motorneurons, we turned to computational modeling and simulations.

To preliminarily test our hypotheses about the mechanism for adaptive change in excitability properties, we adopted a detailed, conductance-based model of a cortical pyramidal cell developed in NEURON and available in the ModelDB database (Accession# 114394; http://senselab.med.yale.edu/ModelDB) in which AIS and axonal passive and active properties were tuned based on direct electrophysiological recordings from the axon and soma that are not currently available for spinal motoneurons (Fig. 5A) [28]. In the model, a 4-fold global increase in the maximal M-channel conductance density, in conjunction with a few millivolts hyperpolarization, reduced changes in membrane potential induced by depolarizing and hyperpolarizing current injections to the soma from a common starting potential (Fig. 5B) in a manner similar to how high K+ treatment alters spinal motoneuron intrinsic excitability. In recording from the soma of hippocampal neurons, pharmacological inhibition of M-channels has been shown to cause a hyperpolarization of the action potential (AP) threshold [29,30], while cortical pyramidal neurons, electrophysiological recordings in the axon have shown that M-channel inhibition causes a depolarization of the action potential threshold at the AIS, with no change of the somatic action potential voltage threshold [31].

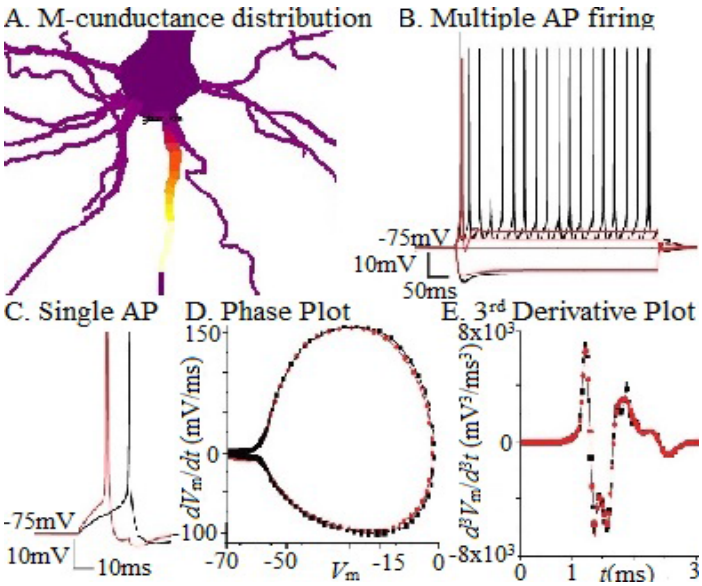

Figure 5. Global M-channel conductance increase reduces the excitability of a neuron model with detailed AIS properties, but does not modify AIS or somatodendritic voltage thresholds. **A**. M-conductance distribution in the somatodendritic and AIS regions of the model (purple→yellow: low to high conductance density). B-C: Representative responses of the model to similar current injections with (red) and without (black) a 4-fold increase in the maximal M-conductance density. D-E. First (D) and third (E) time derivative plots of the model membrane potential as a function of the membrane potential and time, respectively with (red) and without (black) a 4-fold increase in the maximal M-conductance density.

In our adapted model, a 4-fold increase in the maximal M-channel conductance density negligibly modified the single action potential properties in the soma (Fig. 5 C-E). This suggests that the alteration in the somato-dendritic and AIS properties of spinal motoneurons after high K+ treatment likely involves a more complex modulation than a simple increase in the conductance of M-channels.

In our electrophysiological experiments, sustained spinal network activation appeared to affect the somato-dendritic region and the AIS of motoneurons differently - hyperpolarizing the voltage threshold of the somato-dendritic region (i.e. increased excitability) while depolarizing the threshold in the AIS (i.e. decreased excitability; Fig. 3). Recent work in CNS neurons has demonstrated that the AIS can be modified – that a neuron's recent activity can change the size of the AIS and its relative location to the soma, as well as altering its ion channel expression [5,32,33]. In dissociated hippocampal cultures, chronic depolarization with high extracellular K+ shifts the AIS ~17 μm away from the soma, causing a decrease in cell excitability [34].

We wondered whether a similar effect could underlie the variability motoneurons treated with high K+. With a combination of IHC and electrophysiological techniques, we can experimentally distinguish changes in somatodendritic and AIS responsiveness and morphological properties of motoneurons that will allow us to understand the complex effects of prolonged activation on motoneuron excitability (Fig. 6, next page)

However first, in order to test the potential for changes in the AIS to explain the changes in electrical properties that we observe in motor neurons, we turned to computational modeling. In our adapted model we modified the compartmentalization of the AIS, dividing it into three ~17 μm-long compartments – (which is actually more in line with the AIS length measured in neonatal spinal motoneurons; Fig. 6B). We then redistributed the active conductances into one of the compartments, either proximal or distal. In addition, we adjusted the maximal conductance density of each AIS conductance according to the change in the total surface from the original model to one of the two newly generated ones (Fig.7, next page). This was done to compensate for the smaller area of the AIS and the lower conductance produced by reducing the AIS length or moving it more proximally which, because of the AIS proximal-to-distal tapering, would result in a reduced AIS dimension, as well.

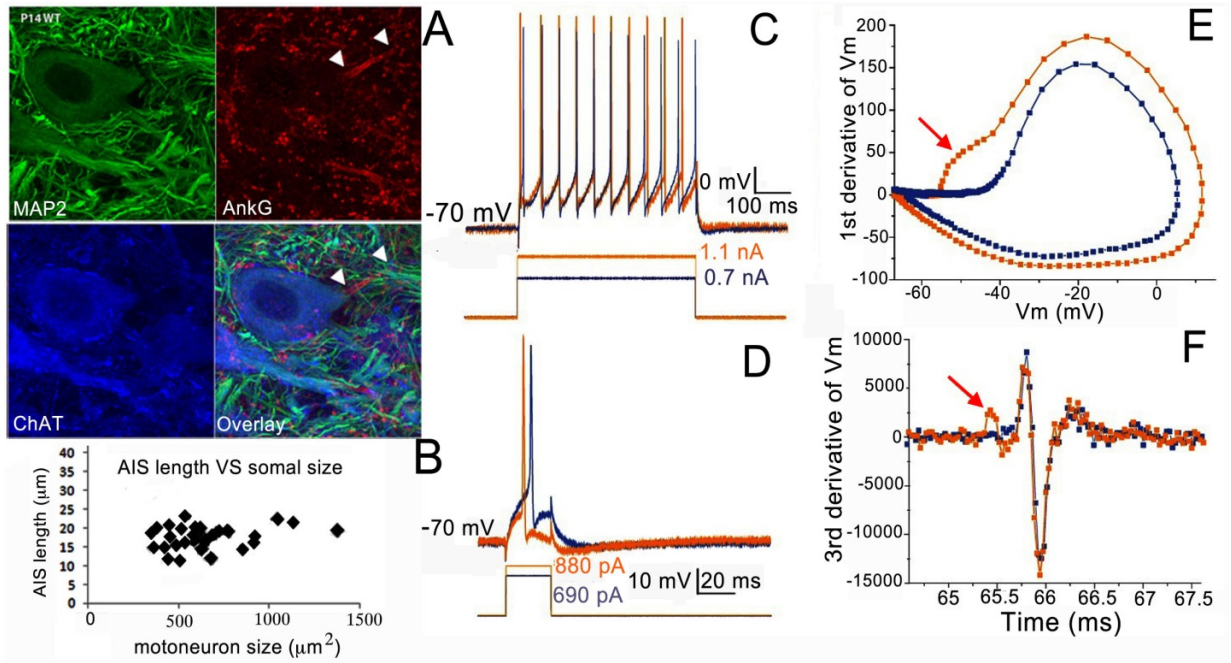

Figure 6. Axon initial segment in motoneurons can be distinguished morphologically and electrically
A. Confocal images of IHC of AIS in spinal motoneurons labeling MAP2 (green) to show axons, AnkyrinG (red) to identify the AIS and ChAT (blue) to label motoneurons. **B**. Quantification of somal size and AIS length in 39 motoneurons. **C-D.** Representative responses at a common membrane potential (– 70 mV) for 2 motoneurons recorded from slices incubated in high (10 mM) K^+ for 30 min. **C.** 500 ms current injections. **D.** Minimal supra-threshold 25 ms current pulse. **E.** Phase plot of the first time derivative of the membrane potential as a function of the membrane potential showing the biphasic rising phase of a motoneuron action potential (orange) with distinguishable AIS (earlier, red arrow) and somato-dendritic (later) components. **D.** Third time derivative of the membrane potential as a function of time showing the presence of an initial peak (red arrow) in the motoneuron (orange) with a distinguishable AIS and somatodendritic components and its absence in the motoneuron with undistinguishable action potential components (blue) [2].

As expected, the model with more distal AIS required more injected current to elicit single or multiple APs (Fig. 7 D-E, next page). However, no change in the resting membrane potential or input resistance of the model was observed. Interestingly, both the phase plot and the third derivative plot revealed some profound differences in the AP properties when the location of the AIS changes (Fig. 7F-G). In particular, the model with more distal AIS generated a non-monotonous trend in the phase plot as well as additional early peaks in the 3rd derivative plot which were not generated by the more proximal AIS model (Fig. 7 F-G). The differences between the more proximal and more distal AIS models resembled our experimental results, though in an exaggerated fashion. Thus, the modeling suggests that the high K+ treatment of spinal motoneurons might alter the location of the AIS relative to the soma.

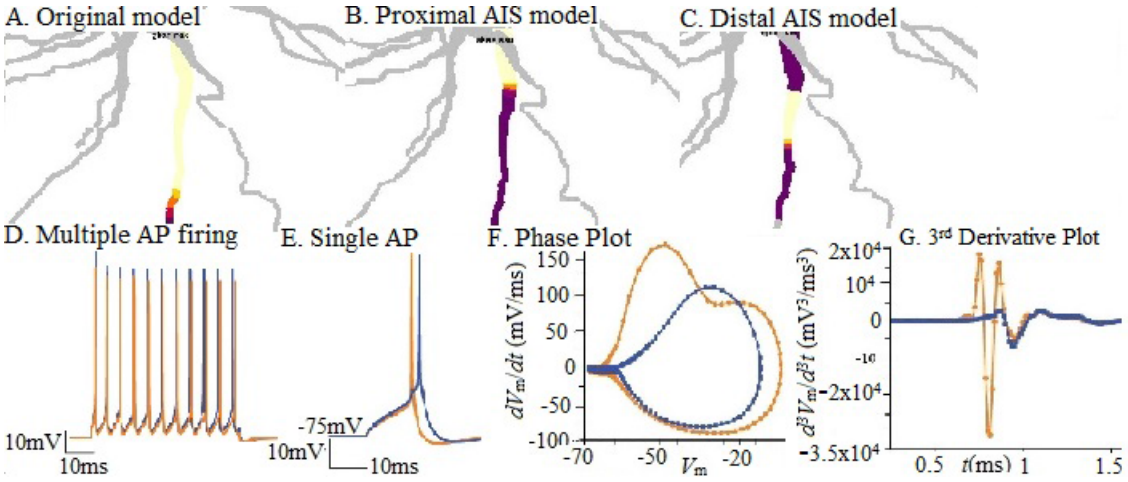

Figure 7. Shifts in AIS location modify thresholds but no other intrinsic excitability properties. A-C. Distribution of the axonal Na conductance (purple→yellow: low to high conductance density) in the original model (A). or in the modified models with a more proximal (B) or more distal (C) AIS. **D-E.** Representative responses from a common membrane potential (– 75 mV) of the model with a more proximal (blue) or distal (orange) AIS to either (D) 500 ms or (E) 25 ms current injections. **F-G.** First (F) and third (G) time derivative plots of the model membrane potential as a function of its membrane potential or time, respectively.

Our modeling experiments point to the potential involvement of changes in the AIS in altering excitability, Our modeling experiments show that shifting the AIS distally from the motoneuron soma will produce changes in neuronal excitability consistent with what we observe after persistent network activation, suggesting the need for more investigation of the biological system. We are now working on immunohistochemical, electrophysiological and modeling techniques to distinguish changes in somatodendritic and AIS responsiveness and morphological properties of motoneurons that result from prolonged activation. We will investigate whether these changes in excitability we observe with network activation are primarily due to sustained activation of excitatory synaptic inputs to motoneurons, or sustained firing of the motoneurons themselves, or a combination of the two. Our integration of biological measurements and computational modeling will allow us to understand the complex effects of prolonged activation on motoneuron excitability, to develop and test hypotheses about the molecular mechanisms of modulation of intrinsic properties in motoneurons, and make predictions generalizable to other neuronal types.

Relationship to Army mission:

Understanding the basic rules for how neurons respond to changing patterns of inputs with a varying pattern of action potential output represents a central goal in neuroscience. Mechanisms of synaptic plasticity have been intensively studied in the central nervous system, but the potential for plasticity in neurons' intrinsic properties has received little attention. Adaptive modulation of the intrinsic excitability of spinal motoneurons has been shown to occur in motor learning [1,5], fast to slow α-motoneuron type conversion [32], and central fatigue [18], while maladaptive modulation may be a characteristic of motoneuron disorders such as SMA and ALS [33,34]. However, the mechanisms for activity-dependent plasticity changes in spinal motoneurons are not understood, and to date, no molecular pathway has been identified that limits their output over the timeframe of minutes to hours.

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