

Wright State University

## CORE Scholar

---

Neuroscience, Cell Biology & Physiology Faculty  
Publications

Neuroscience, Cell Biology & Physiology

---

6-30-2021

# The Role of Action Potential Waveform in Failure of Excitation Contraction Coupling

Xueyong Wang

Wright State University - Main Campus, xueyong.wang@wright.edu

Murad Nawaz

Wright State University, nawaz.3@wright.edu

Steve R.A. Burke

Wright State University - Main Campus, steven.burke@wright.edu

Roger Bannister

Brent D. Foy

Wright State University - Main Campus, brent.foy@wright.edu

*See next page for additional authors*

Follow this and additional works at: <https://corescholar.libraries.wright.edu/ncbp>



Part of the [Medical Cell Biology Commons](#), [Medical Neurobiology Commons](#), [Medical Physiology Commons](#), [Neurosciences Commons](#), and the [Physiological Processes Commons](#)

---

### Repository Citation

Wang, X., Nawaz, M., Burke, S. R., Bannister, R., Foy, B. D., Voss, A. A., & Rich, M. M. (2021). The Role of Action Potential Waveform in Failure of Excitation Contraction Coupling. *bioRxiv*.  
<https://corescholar.libraries.wright.edu/ncbp/1183>

This Article is brought to you for free and open access by the Neuroscience, Cell Biology & Physiology at CORE Scholar. It has been accepted for inclusion in Neuroscience, Cell Biology & Physiology Faculty Publications by an authorized administrator of CORE Scholar. For more information, please contact [library-corescholar@wright.edu](mailto:library-corescholar@wright.edu).

---

**Authors**

Xueyong Wang, Murad Nawaz, Steve R.A. Burke, Roger Bannister, Brent D. Foy, Andrew A. Voss, and Mark M. Rich

1 Title: The Role of Action Potential Waveform in Failure of Excitation Contraction Coupling

2 Running title: Action Potentials and Excitation Contraction Coupling

3

4 Xueyong Wang<sup>1</sup>, Murad Nawaz<sup>1</sup>, Steve R.A. Burke<sup>2</sup>, Roger Bannister<sup>3</sup>, Brent D. Foy<sup>4</sup>, Andrew  
5 A. Voss<sup>2</sup>, Mark M. Rich<sup>1</sup>

6

7 <sup>1</sup>Wright State University, Department of Neuroscience, Cell Biology, and Physiology, 143

8 Biological Sciences II, 3640 Colonel Glenn Hwy, Dayton, OH 45435, USA

9 <sup>2</sup>Wright State University, Department of Biological Sciences, 235 Biological Sciences, 3640

10 Colonel Glenn Hwy, Dayton, OH 45435, USA

11 <sup>3</sup>Roger A. Bannister, PhD, Departments of Pathology/Biochemistry & Molecular Biology,

12 University of Maryland School of Medicine, 108 North Greene Street, Baltimore, MD, 21201

13 USA

14 <sup>4</sup>Wright State University, Department of Physics, 248 Fawcett Hall, 3640 Colonel Glenn Hwy,

15 Dayton, OH 45435, USA

16

17 Corresponding author: Mark Rich, ORCID: [mark.rich@wright.edu](mailto:mark.rich@wright.edu)

18 Author contributions: XW, MN and SRAB contributed to acquisition, analysis or interpretation

19 of data. RB, BDF, AAV and MMR contributed to drafting the work or revising it critically for

20 important intellectual content. All authors approved of the final manuscript and take

21 responsibility for the work.

22

23 Key words: action potential, excitation contraction coupling, resting potential, depolarization,

24 calcium release

25 Funding: This work was supported by NIH grant AR074985 (M.M.R.) and MDA grant 602459

26 (M.M.R.)

27

28 Abstract:

29 Excitation contraction coupling (ECC) is the process by which electrical excitation of muscle is  
30 converted into force generation. Depolarization of skeletal muscle resting potential contributes  
31 to failure of ECC in diseases such as periodic paralysis, ICU acquired weakness and possibly  
32 fatigue of muscle during vigorous exercise. When extracellular  $K^+$  is raised to depolarize the  
33 resting potential, failure of ECC occurs suddenly, over a range of several mV of resting potential.  
34 While some studies have hypothesized the sudden failure of ECC is due to all-or-none failure of  
35 excitation, other studies suggest failure of excitation is graded. Intracellular recordings of action  
36 potentials (APs) in individual fibers during depolarization revealed that APs do not fail in an all-  
37 or-none manner. Simultaneous imaging of  $Ca^{2+}$  transients during depolarization revealed failure  
38 over a narrow range of resting potentials. An AP property that closely correlated with the  
39 sudden failure of the  $Ca^{2+}$  transient was the integral of AP voltage with respect to time. We  
40 hypothesize the close correlation is due to the combined dependence on time and voltage of  $Ca^{2+}$   
41 release from the sarcoplasmic reticulum. The quantitative relationships established between  
42 resting potential, APs and  $Ca^{2+}$  transients provide the foundation for future studies of  
43 depolarization-induced failure of ECC in diseases such as periodic paralysis.

44

45

46 Introduction:

47 The process by which electrical excitation of muscle is converted into force generation is  
48 known as excitation contraction coupling (ECC). Successful ECC involves invasion of action  
49 potentials into a network of membrane invaginations in muscle known as the transverse tubules  
50 (t-tubules) (Adrian et al., 1969). Depolarization in the t-tubules activates Cav1.1 channels,  
51 which triggers opening of ryanodine receptors, Ca<sup>2+</sup> exit from the sarcoplasmic reticulum and  
52 force production (Melzer et al., 1995; Dulhunty, 2006; Bannister and Beam, 2013; Hernandez-  
53 Ochoa and Schneider, 2018).

54 Depolarization of the resting membrane potential of skeletal muscle, when severe  
55 enough, causes failure of ECC in diseases such periodic paralysis and ICU acquired weakness  
56 (Lehmann-Horn et al., 2008; Cannon, 2015; Friedrich et al., 2015) as well as potentially  
57 contributing to fatigue during intense exercise (Allen et al., 2008). Studies of depolarization-  
58 induced failure of ECC in frog and mammalian skeletal muscle are consistent with all-or-none  
59 failure of force generation in individual fibers (Renaud and Light, 1992; Cairns et al., 1997;  
60 Cairns et al., 2011). Whole muscle force is generally stable or slightly increased with mild  
61 depolarization of the resting potential, which is followed by a steep decline with further  
62 depolarization by only a few mV (Holmberg and Waldeck, 1980; Renaud and Light, 1992;  
63 Cairns et al., 1997; Yensen et al., 2002; Cairns et al., 2011; Pedersen et al., 2019). The sudden  
64 decrease in force is paralleled by a decrease in the Ca<sup>2+</sup> transient with depolarization of the  
65 resting potential beyond -60 mV (Quinonez et al., 2010).

66 While the idea of all-or-none muscle contraction dates back more than 100 years (Pratt,  
67 1917), the mechanism underlying the near all-or none failure of force generation in the setting of  
68 depolarization of the resting potential remains unknown. One hypothesis is that the sudden  
69 failure of ECC is due to all-or-none failure of excitability (Renaud and Light, 1992; Cairns et al.,  
70 1997). Support for failure of excitability as the mechanism comes from studies showing that the  
71 decline in force is paralleled by reduction in extracellular recordings of compound muscle action  
72 potentials (Overgaard et al., 1999; Overgaard and Nielsen, 2001; Pedersen et al., 2003).  
73 However, intracellular recordings demonstrate graded failure of excitation with depolarization of  
74 the resting potential, which manifests as either a gradual reduction in action potential (AP) peak  
75 or as APs with variable amplitude that increase with increased current injection (Rich and Pinter,  
76 2001, 2003; Quinonez et al., 2010; Ammar et al., 2015; Miranda et al., 2020; Uwera et al., 2020).

77 Two studies have suggested reduction in AP peak can cause reduction in force generation  
78 (Cairns et al., 2003; Gong et al., 2003). These studies are consistent with graded failure of force  
79 production in individual fibers.

80 To determine the mechanism underlying the sudden failure of ECC, we measured muscle  
81 force, APs and  $\text{Ca}^{2+}$  transients in muscle in which the resting potential was depolarized by  
82 elevation of extracellular  $\text{K}^+$ . In agreement with previous studies, mouse EDL twitch force  
83 decreased dramatically over a narrow range of extracellular  $\text{K}^+$  concentrations (Cairns et al.,  
84 1997; Yensen et al., 2002). Intracellular recording of APs, combined with  $\text{Ca}^{2+}$  imaging in  
85 individual fibers during elevation of extracellular  $\text{K}^+$ , revealed graded failure of excitation was  
86 accompanied by relatively sudden failure of the intracellular  $\text{Ca}^{2+}$  transient. We identified an AP  
87 parameter that closely correlated with failure of the  $\text{Ca}^{2+}$  transient: the area of the AP above -30  
88 mV. Identification of this unexpectedly accurate predictor of failure of the  $\text{Ca}^{2+}$  transient  
89 provides a basis for future studies of depolarization-induced failure of ECC.

90

91

92 Methods:

93 *Mice:*

94 All animal procedures were performed in accordance with the policies of the Animal  
95 Care and Use Committee of Wright State University and were conducted in accordance with the  
96 United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals.  
97 Mice expressing GCAMP6f (Chen et al., 2013) in skeletal muscle were generated by crossing  
98 floxed GCAMP6f mice (Jackson Labs, B6J.Cg-*Gt(ROSA)26Sortm95.1(CAG-*  
99 *GCaMP6f)Hze/MwarJ*, cat #028865) with mice expressing parvalbumin promoter driven Cre  
100 (Jackson Labs, B6.129P2-Pvalbtm1(*cre*)Arbr/J, cat# 030218).

101

102 *Ex vivo force measurements.*

103 Mice were euthanized by inhalation of a saturating dose of isoflurane (~2 g/L) followed  
104 by cervical dislocation. The hind limb *extensor digitorum longus* (EDL) muscle was dissected  
105 and the proximal tendon of the EDL was tied with a 6-0 caliber silk suture to a bar attached to a  
106 custom recording chamber. The distal tendon was tied to a hook and attached to the force  
107 transducer (Aurora Scientific). Force measurements were recorded at 21–23°C with the EDL  
108 submerged in a solution containing (in mM) 118 NaCl, 3.5 KCl, 1.5 CaCl<sub>2</sub>, 0.7 MgSO<sub>4</sub>, 26.2  
109 NaHCO<sub>3</sub>, 1.7 NaH<sub>2</sub>PO<sub>4</sub>, and 5.5 glucose and maintained at pH 7.3-7.4 by aeration with 95% O<sub>2</sub>  
110 and 5% CO<sub>2</sub>. Solutions containing elevated concentrations of KCl (3.5, 10, 12, 14, and 16 mM)  
111 and with corresponding reduction in NaCl (118, 111.5, 109.5, and 105.5 mM respectively) to  
112 maintain a constant osmolarity were used to induce depolarization. The EDL was stimulated with  
113 two electrodes placed perpendicularly to the muscle in the bath. The force transducer was  
114 controlled by a 305C two-channel controller (Aurora Scientific) and digitized by a Digidata  
115 1550B digitizer (Molecular Devices). A S-900 pulse generator (Dagan) was used to generate 0.1  
116 ms 5V twitch stimulations to the muscle. The pulse generator was triggered using pCLAMP 11  
117 data acquisition and analysis software. The optimal length was determined by adjusting the  
118 tension of the muscle until maximal twitch force was achieved. During force recordings, the  
119 muscle was exposed to normal K<sup>+</sup> solution for 20 minutes, followed by high K<sup>+</sup> solution (10 mM  
120 to 16mM) for 45 minutes, and then washed again with normal K<sup>+</sup> solution for 25 minutes to  
121 follow recovery. The EDL was stimulated with a twitch pulse every 5 minutes, and force was  
122 recorded.

123

124 *Ex vivo recordings of action potentials and Ca<sup>2+</sup> transients.*

125 Mice were sacrificed using CO<sub>2</sub> inhalation followed by cervical dislocation, and both  
126 extensor digitorum longus (EDL) muscles were dissected out tendon-to-tendon. Muscles were  
127 maintained and recorded at 22°C within 6 hours of sacrifice. Standard solution contained (in  
128 mM): 118 NaCl, 3.5 KCl, 1.5 CaCl<sub>2</sub>, 0.7 MgSO<sub>4</sub>, 26.2 NaHCO<sub>3</sub>, 1.7 NaH<sub>2</sub>PO<sub>4</sub>, 5.5 glucose, and  
129 maintained at pH 7.3-7.4 by aeration with 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

130 To prevent contraction, muscles were loaded with 50μM BTS (N-benzyl-p-  
131 toluenesulfonamide, Tokyo Chemical Industry, Tokyo, Japan, catalogue #B3082) dissolved in  
132 DMSO for 45 minutes prior to recording.

133 Muscle fibers were impaled with 2 sharp microelectrodes filled with 2 M potassium  
134 acetate solution containing 1 mM sulforhodamine 101 (Sigma-Aldrich, Catalogue #S7635) to  
135 allow for visualization. Resistances were between 25 and 50 MΩ, and capacitance compensation  
136 was optimized prior to recording. APs were evoked by a 0.2 ms injection of current. Fibers with  
137 resting potentials more depolarized than -74 mV in solution containing 3.5 mM KCl were  
138 discarded.

139

140 *Imaging of Ca<sup>2+</sup> transients.*

141 Muscle expressing GCAMP6f was imaged without staining (LeiCa<sup>2+</sup> I3 cube, band pass  
142 450-490, long pass 515). Imaging was synchronized with triggering of APs using a Master-8  
143 pulse generator (A.M.P.I.). Frames were acquired at 30 frames per second with a sCMOS  
144 camera (CS2100M-USB) using ThorCam software (Thorlab Inc. NJ). During infusion of  
145 solution containing high K, APs were triggered every 5s. Each AP was synchronized with  
146 capture of 48 frames at 30 frame/s. Images were analyzed using Image J (NIH). Regions of  
147 interest (ROI) were set both on the muscle fiber being stimulated and a neighboring fiber. The  
148 neighboring fiber was used to record background, which was subtracted from the ROI on the  
149 stimulated fiber.

150

151 *Fitting of data with Boltzmann distributions.*

152 Data for AP peak vs. resting potential, Ca<sup>2+</sup> image intensity vs. AP, and Ca<sup>2+</sup> image  
153 intensity vs. resting potential were all fit to a Boltzmann,



154

155 
$$Out = LV + \frac{(HV-LV)}{1+e^{\frac{(V50-V)}{k}}} \quad \text{Eq. 1}$$

156

157 where *Out* represents the dependent variable (either AP peak or Ca<sup>2+</sup> image intensity), *V* is the  
158 independent voltage variable (either resting potential or AP peak), *LV* is the limiting value when  
159 *V* is very low (toward more negative), *HV* is the limiting value when *V* is very high (toward more  
160 positive), *V50* is the value of *V* at which *Out* is halfway between *HV* and *LV*, and *k* is the slope  
161 factor. All voltages and the variable *k* are expressed in mV, and Ca<sup>2+</sup> image intensity is in  
162 arbitrary units between 1 for maximum intensity for each experiment and 0.

163

#### 164 *Statistics.*

165 Data for recordings from different muscles were analyzed using nested analysis of  
166 variance with *n* as the number of mice, with data presented as mean ± SD. Comparisons of  
167 different parameters recorded from the same fiber were compared using the paired students t-test.  
168 *p* < 0.05 was considered to be significant. The numbers of animals and fibers for comparisons are  
169 described in the corresponding figure legends and text.

170

#### 171 *Results:*

172 Measurement of twitch force in the mouse EDL following elevation of extracellular K<sup>+</sup> to  
173 between 10 and 16 mM yielded results similar to those previously reported. There was an initial  
174 increase in force (Fig 1A, *n* = 3 muscles for each K<sup>+</sup> concentration) (Cairns et al., 1997; Yensen  
175 et al., 2002; Pedersen et al., 2019). The initial increase in force is paralleled by an increase in the  
176 Ca<sup>2+</sup> transient, which occurs despite a reduction of AP peak (Quinonez et al., 2010; Pedersen et  
177 al., 2019). The mechanism underlying the increase in Ca<sup>2+</sup> transient remains unknown; it does  
178 not appear to be due to changes in characteristics of APs (Yensen et al., 2002).

179

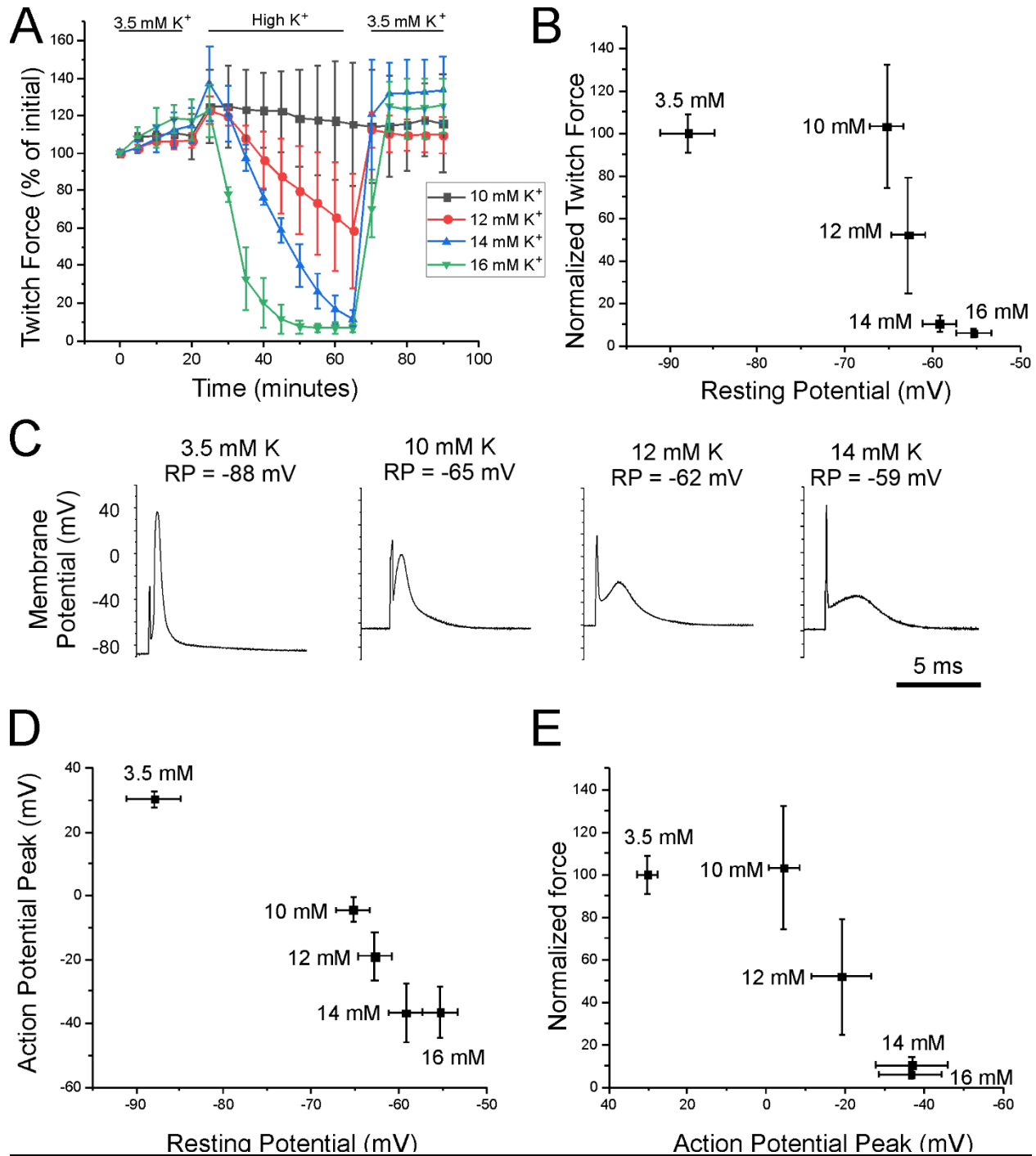
180 Following the initial increase in force, there was a decline that became faster with higher  
181 levels of extracellular K<sup>+</sup> (Fig 1A). With return to solution containing normal K<sup>+</sup>, force  
182 recovered rapidly. Force generated 40 minutes following infusion of high K<sup>+</sup> was steeply  
183 dependent on extracellular K<sup>+</sup>, such that force was near normal in 10 mM K<sup>+</sup> but near 0 in 14 and  
184 16 mM K<sup>+</sup>. The mean resting potential 20-40 minutes after infusion of each concentration of K<sup>+</sup>

184 was measured in a separate set of experiments ( $n = 80$  fibers from 4 muscles for each  $K^+$   
185 concentration) and those data were used to construct a plot of force versus mean resting  
186 potential. There was a steep loss of force over a narrow range of resting potentials: normal force  
187 was generated at a mean resting potential of  $-65.3 \pm 1.9$  mV in 10 mM  $K^+$  and almost no force  
188 was generated at a mean resting potential of  $-59.3 \pm 1.9$  mV in 14 mM  $K^+$  (Fig 1B). Our finding  
189 is similar to a previous report of steep dependence of force production on resting potential  
190 (Cairns et al., 1997).

191 The sudden loss of force with depolarization of muscle has been hypothesized to be due  
192 to all-or-none failure of APs (Renaud and Light, 1992; Cairns et al., 1997). To test this  
193 hypothesis, we measured intracellular APs from EDL fibers in each of the high  $K^+$  solutions.  
194 APs were measured with a voltage-sensing electrode and triggered with a current-passing  
195 electrode. While APs became smaller with depolarization, responses were still present in 14  
196 and 16 mM  $K^+$ ; concentrations of  $K^+$  in which force production is near 0 (Fig 1B, C). These data  
197 counter the idea of all-or-none APs, and agree with several studies suggesting more gradual  
198 failure of excitation (Rich and Pinter, 2001, 2003; Quinonez et al., 2010; Ammar et al., 2015;  
199 Miranda et al., 2020; Uwera et al., 2020).

200 If depolarization of the resting potential triggers a gradual decline of APs, why is there  
201 close to all-or-none failure of force production with depolarization? One possibility is that small  
202 APs fail to trigger elevation of intracellular  $Ca^{2+}$ . At resting potentials between -80 and -90 mV,  
203 APs peaked at  $30.2 \pm 2.7$  mV. With depolarization of the resting potential to  $-65.3 \pm 1.9$  mV, the  
204 peak was reduced to  $-4.4 \pm 3.9$  mV and at a resting potential of  $-55.4 \pm 2.0$  mV, the peak  
205 averaged  $-36.5 \pm 8.0$  mV (Fig 1D). Reduction of the mean AP peak from 30.2 mV to -4.4 mV  
206 was associated with little to no reduction in force, whereas reduction of the peak from -4 mV to -  
207 36 mV was associated with almost complete loss of force (Fig 1 E). These data raise the  
208 possibility that there may be a threshold for AP peak above which APs trigger full contraction  
209 and below which there is failure of ECC.

210



211

212 Figure 1: Loss of force secondary to depolarization of the resting potential is accompanied by  
213 graded failure of excitation. A) Shown is a plot of extensor digitorum longus (EDL) twitch force  
214 versus time following infusion of various concentrations of external K<sup>+</sup> (n = 3 muscles for each  
215 K<sup>+</sup> concentration, error bars = SD). Force for each muscle was normalized to the initial force  
216 recorded. B) Mean force after 40 minutes in high K<sup>+</sup> (from Fig 1A) normalized to force after 40

217 minutes in 3.5 mM  $K^+$  plotted versus mean resting membrane potential recorded 20 to 40  
218 minutes following infusion of high  $K^+$  solution. C) Examples of typical APs recorded at various  
219 resting membrane potentials. In each trace the AP is preceded by a 0.2 ms stimulus artifact. D)  
220 Plot of mean AP peak versus mean resting potential for each of the  $K^+$  concentrations. E) Plot of  
221 mean force 40 minutes following elevation of  $K^+$  versus mean AP peak for each  $K^+$   
222 concentration.

223

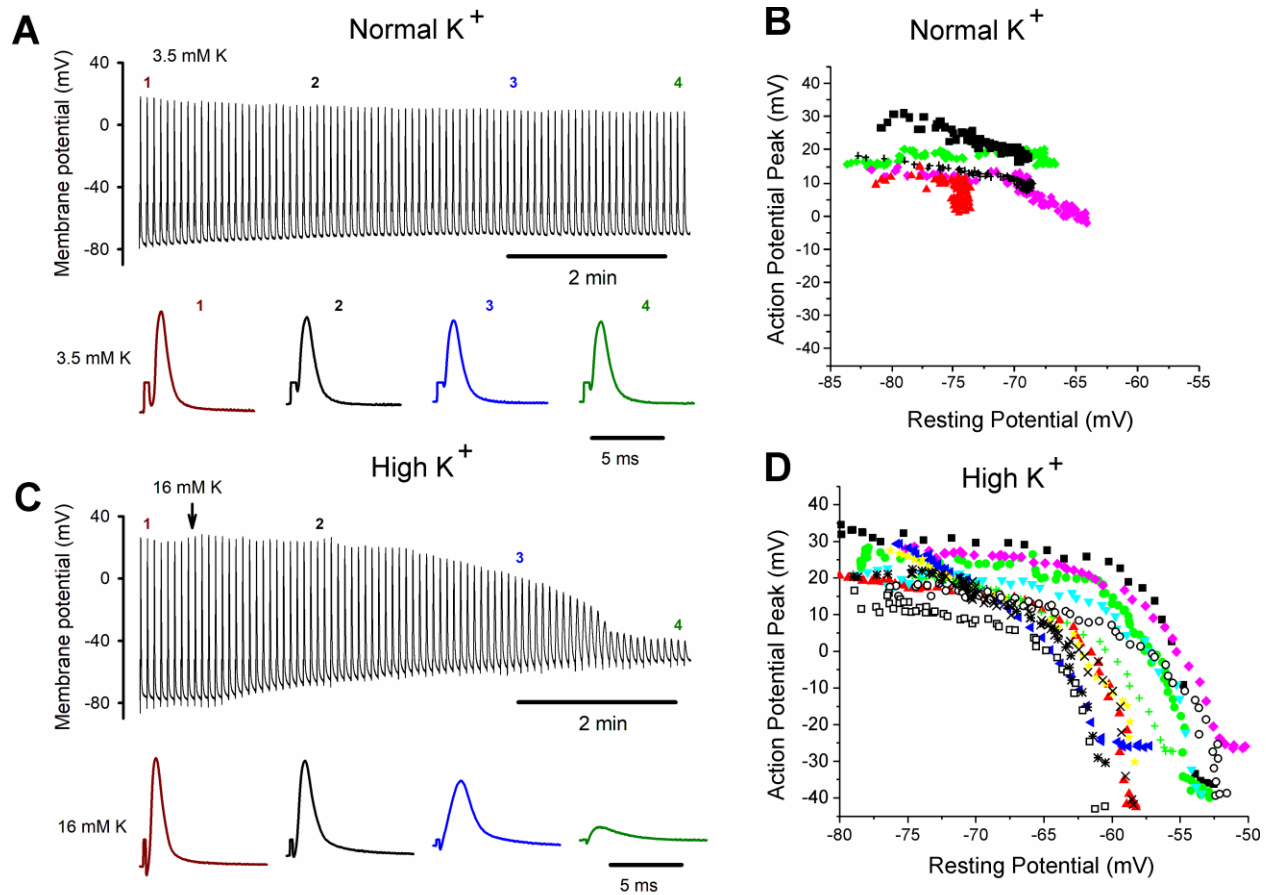
224

225 The relationship between depolarization of the resting potential and reduction in AP peak  
226 has never been studied in individual fibers. To determine the contribution of damage-induced  
227 depolarization to AP changes during prolonged impalement, we performed 7-minute recordings  
228 of resting potential and AP peaks in solution containing normal external  $K^+$ . Seven minutes after  
229 impalement, impalement-induced damage caused 8 to 20 mV of depolarization of the resting  
230 membrane potential (Fig 2 A, B), which was accompanied by up to 15 mV of reduction in AP  
231 peak (Fig 2 B). Infusion of a solution containing 16 mM  $K^+$  causes substantial depolarization of  
232 the resting membrane potential and reduction in the AP peak beyond that seen with impalement  
233 alone (Fig 2 C, D). Consistent with recordings taken from populations of fibers, infusion of 16  
234 mM  $K^+$  caused graded reduction of the AP peak from a maximum ranging from +15 to +35 mV  
235 down to -25 to -45 mV. Between resting potentials of -65 to -52 mV, there was rapid reduction  
236 of the peak with further depolarization. To quantitate the relationship between depolarization of  
237 the resting potential and reduction in AP peak, we fit the data for individual fibers (plots in Fig  
238 2D) with Boltzmann equations (Eq. 1, See Fig 3G for an example fit). For these fits, the HV  
239 limit, which represents the minimal AP peak when resting potential was elevated, was  
240 constrained to be between -30 mV and -50 mV. The  $V_{50}$  for the resting potential at which AP  
241 peak was half maximal was  $-58.2 \pm 3.3$  mV, the slope factor  $k$  was  $1.8 \pm 0.6$  mV, and the average  
242 value of the half-maximal AP peak at the  $V_{50}$  value was  $-15.5 \pm 4.9$  mV ( $n = 12$  fibers from 6  
243 mice).

244

245

246



247

248 Fig 2: Failure of excitation in individual fibers during depolarization. A) Top: Shown are APs  
249 from a fiber during a 7-minute recording in 3.5 mM K<sup>+</sup>. APs were triggered at 0.2 Hz by a 0.2  
250 ms injection of depolarizing current of constant amplitude that was 150% of initial threshold  
251 current. The recording is not continuous: a 5 ms block of time is shown for each AP and  
252 stimulus artifacts have been removed. The time base indicated is for the time between APs.  
253 Bottom: Shown on an expanded time base are APs from the top traces at the time points  
254 indicated by the numbers. Stimulus artifacts have been truncated for clarity. B) Plot of AP peak  
255 versus resting potential during for 5 fibers in which K<sup>+</sup> was maintained at 3.5 mM throughout the  
256 7-minute recording. The impalement-induced depolarization in the 5 fibers ranged from 8 to  
257 close to 20 mV. C) Top: An intracellular recording from a fiber during infusion of 16 mM K<sup>+</sup>.  
258 The infusion began at the time indicated by the vertical arrow. Bottom: Shown on an expanded  
259 time base are APs from the time points indicated by the numbers. D) Plot of AP peak versus  
260 resting potential during infusion of 16 mM K<sup>+</sup> for 12 fibers.

261

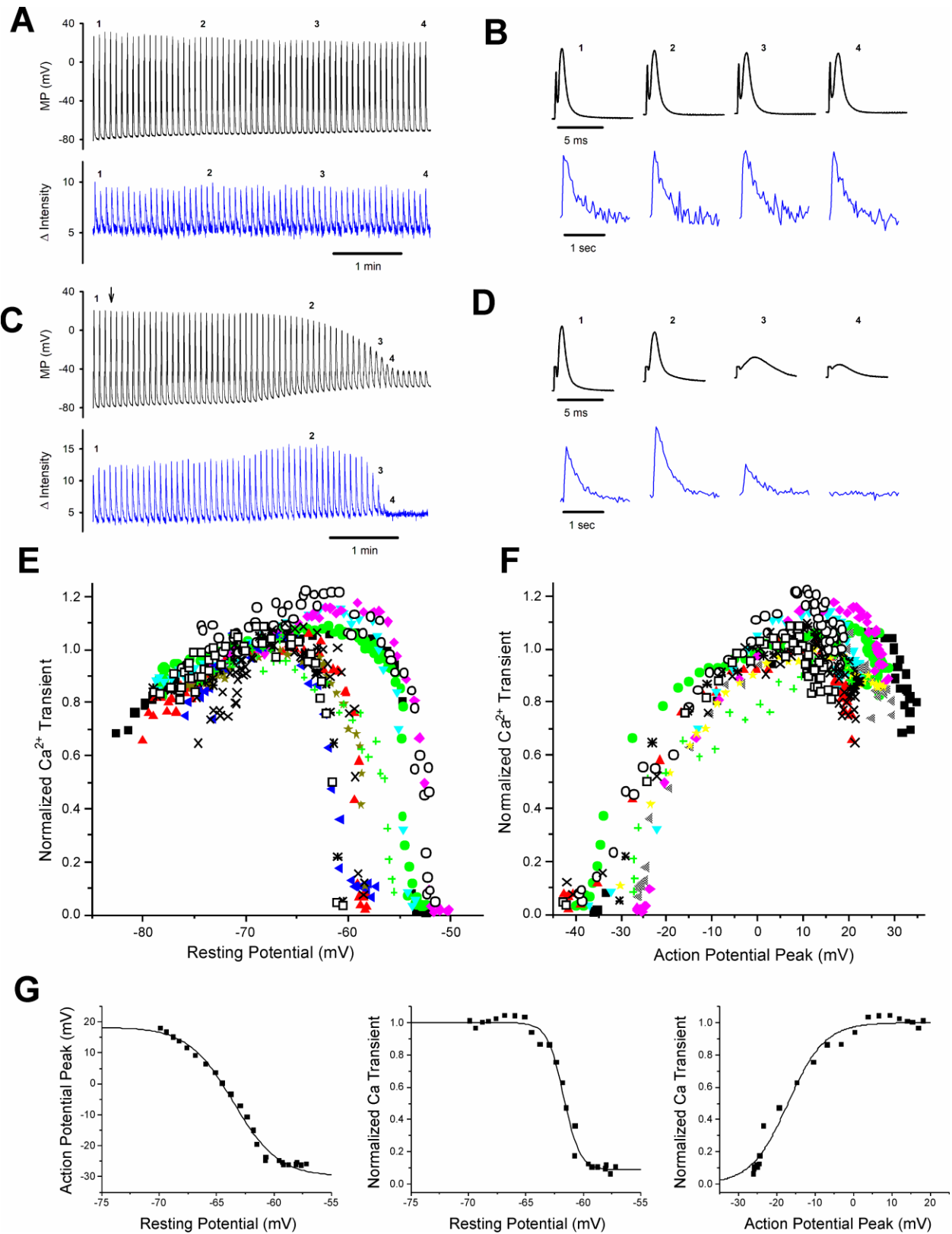
262

263 In order to better understand how depolarization of the resting potential affects generation  
264 of force, we recorded APs while simultaneously imaging  $\text{Ca}^{2+}$  transients in fibers from mice  
265 expressing GCAMP6f in skeletal muscle. GCAMP6f is a high affinity genetically-encoded  $\text{Ca}^{2+}$   
266 indicator which allowed us to determine changes in amplitude of the  $\text{Ca}^{2+}$  transient even during  
267 low release flux events, but which does not allow for examination of kinetics (Chen et al., 2013;  
268 Shang et al., 2014; Dana et al., 2019). In normal extracellular  $\text{K}^+$ , single APs generated a bright  
269 fluorescent transient (Fig 3A-D, video associated with this submission). When extracellular  $\text{K}^+$   
270 was kept at 3.5 mM, the peak of the  $\text{Ca}^{2+}$  transient was stable over time, with a slight trend  
271 towards increasing (Fig 3A, B, 1.16 at 7 minutes vs initial value normalized to 1,  $p = .07$ ,  $n = 5$ ).

272 With depolarization during infusion of 16 mM  $\text{K}^+$ , there was an initial increase in the  
273  $\text{Ca}^{2+}$  transient from a mean normalized value of  $0.81 \pm 0.10$  to the normalized maximum of 1 ( $p$   
274  $< .0001$  paired t-test,  $n = 12$ , Fig 3C-E). This increase is similar to what has been reported  
275 previously (Quinonez et al., 2010; Pedersen et al., 2019) and occurred despite reduction in AP  
276 peak (Fig 3D, see Fig 2D for AP peak plots for the same 12 fibers). As depolarization progressed  
277 there was rapid, complete loss of the  $\text{Ca}^{2+}$  transient (Fig 3C-E). To quantitate the relationship  
278 between depolarization and failure of the  $\text{Ca}^{2+}$  transient we fit the data for the decrease in  $\text{Ca}^{2+}$   
279 transient with depolarization of the resting potential beyond -70 mV with a Boltzmann equation  
280 (Eq. 1, See Fig 3G for an example fit). For these fits, the LV limit, which represents the  $\text{Ca}^{2+}$   
281 image intensity when resting potential was -70 mV, was fixed to 1, and the HV limit was  
282 constrained to be between 0 and 0.1. The resting potential at which  $\text{Ca}^{2+}$  transient was half  
283 maximal was  $-57.5 \pm 3.4$  mV with a slope factor of  $0.4 \pm 0.2$  mV, which was significantly  
284 steeper than the slope for reduction in AP peak ( $p < 1 \times 10^{-5}$ , paired t-test,  $n = 12$  fibers from 6  
285 mice, Fig 3E).

286 We plotted the reduction in  $\text{Ca}^{2+}$  transient versus AP peak (Fig 3F), and fit the data with a  
287 Boltzmann equation (Eq. 1, see Fig 3G for an example fit). For these fits, the HV was fixed to 1  
288 and the LV was fixed to 0. The  $\text{Ca}^{2+}$  transient was half maximal at an AP peak of  $-21.0 \pm 4.0$  mV  
289 with a slope factor of  $5.9 \pm 1.7$  mV. This relationship between peak voltage and  $\text{Ca}^{2+}$  transient  
290 was within the range of values obtained from voltage clamp studies of mouse muscle fibers  
291 (Wang et al., 1999; Gregorio et al., 2017). These data suggest APs peaking below -30 mV  
292 trigger little to no elevation of intracellular  $\text{Ca}^{2+}$  and hence little to no generation of force.

293



294

295

296 Figure 3: Failure of the  $\text{Ca}^{2+}$  transient with depolarization. A) Shown are the AP and  $\text{Ca}^{2+}$   
297 transient for a fiber expressing GCAMP6f in 3.5 mM  $\text{K}^+$  during a recording. The stimulus  
298 artifact in the AP traces has been eliminated for clarity. The recording is not continuous. A 5 ms  
299 block of time is shown for each AP and a 1 s block of time is shown for each  $\text{Ca}^{2+}$  transient. The  
300 time base indicated is for the time between APs and  $\text{Ca}^{2+}$  transients. B) Shown on an expanded  
301 time scale are the APs and corresponding  $\text{Ca}^{2+}$  transients for the 4 time points indicated in A. C)  
302 APs and  $\text{Ca}^{2+}$  transients for a fiber during infusion of solution containing 16 mM  $\text{K}^+$ . D) Shown  
303 on an expanded time scale are APs and corresponding  $\text{Ca}^{2+}$  transients for the 4 time points  
304 indicated in C. E) Plot of the peak of the  $\text{Ca}^{2+}$  transient versus resting potential for the 12 fibers  
305 studied (The same fibers studied in Fig 2). The peak of the  $\text{Ca}^{2+}$  transient present at a resting  
306 potential of -70 mV was normalized to a value of 1 for each fiber. F) Plot of normalized  $\text{Ca}^{2+}$   
307 transient versus AP peak for the 12 fibers studied. G) Shown are example Boltzmann fits of AP  
308 Peak and  $\text{Ca}^{2+}$  transient versus resting potential as well as  $\text{Ca}^{2+}$  transient versus AP peak for a  
309 single fiber.

310

311

312 The finding that all-or none- failure of the AP was not the mechanism underlying  
313 depolarization-induced failure of ECC raises the possibility that measurement of APs might not  
314 allow for accurate prediction of successful ECC. This would necessitate  $\text{Ca}^{2+}$  imaging to  
315 determine the efficacy of APs in triggering ECC. We wished to identify an AP property that  
316 would accurately predict the  $\text{Ca}^{2+}$  transient. We first examined the correlation between  
317 normalized AP amplitude and the  $\text{Ca}^{2+}$  transient. A loss of  $11.6 \pm 1.7$  mV of resting potential  
318 was required to reduce AP amplitude from 90% to 10% of maximum (Fig 4D). In contrast, the  
319  $\text{Ca}^{2+}$  transient was reduced from 90% to 10% of maximum with a loss of only  $4.1 \pm 2.4$  mV of  
320 resting potential (Fig 4D,  $p < 1 \times 10^{-6}$  vs APs,  $n=12$ , paired student's t-test). This statistically  
321 significant difference led us to look for another AP parameter that decreased more sharply with  
322 depolarization of the resting potential.

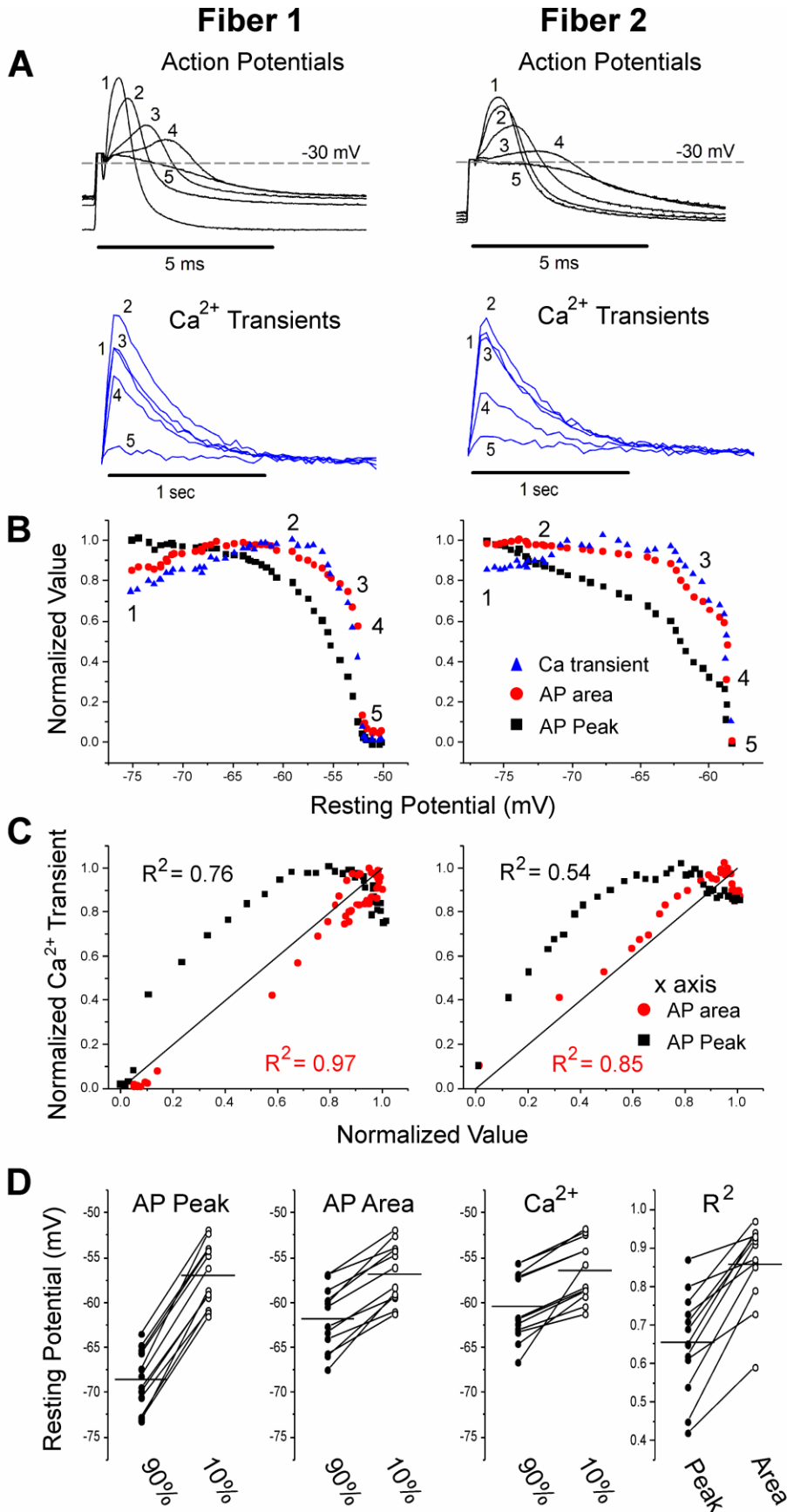
323 As shown in Fig 3F, an AP peak above -30 mV is required to consistently trigger  $\text{Ca}^{2+}$   
324 release. We thus set AP peaks of -30 mV or below to 0 and normalized AP amplitude. At  
325 mildly depolarized resting potentials, drops in normalized AP peaks were accompanied by  
326 increases in the  $\text{Ca}^{2+}$  transient (Fig 4A, B, Fig 3E) (Quinonez et al., 2010; Pedersen et al., 2019).



327 At more depolarized resting potentials, the drop in AP peak did not closely track the sharp drop  
328 in  $\text{Ca}^{2+}$  transient (Fig 4A, B). When the normalized  $\text{Ca}^{2+}$  transient was plotted against the  
329 normalized AP peak, the mean  $R^2$  was  $0.65 \pm 0.14$  (Fig 4C, D,  $n = 12$  fibers). This was not the  
330 close relationship we were hoping to find.

331 We next considered whether changes in AP kinetics might affect the  $\text{Ca}^{2+}$  transient. It  
332 has been shown previously that AP half width increases with depolarization of the resting  
333 membrane potential due to both decreased rate of rise and decreased rate of decay of the AP  
334 (Yensen et al., 2002). It has also been shown previously that increasing AP half width by  
335 blocking  $\text{K}^+$  channels increases twitch force (Delbono and Kotsias, 1987; van Lunteren et al.,  
336 2006), presumably secondary to increases in the  $\text{Ca}^{2+}$  transient. It is thus possible that increases  
337 in AP half width lessen the effect of decreasing AP peak on the  $\text{Ca}^{2+}$  transient during modest  
338 depolarization of the resting potential.

339 To include changes in both AP half width and peak, we measured the integral of AP  
340 voltage with respect to time. The integral above  $-30$  mV was used to account for the lack of  $\text{Ca}^{2+}$   
341 transient when APs peaked below  $-30$  mV. AP area closely paralleled  $\text{Ca}^{2+}$  transient during  
342 depolarization of the resting potential (Fig 4A, B). When the normalized  $\text{Ca}^{2+}$  transient was  
343 plotted against normalized AP area, the mean  $R^2$  value was  $0.86 \pm 0.11$  (Fig 4C, D,  $p < .001$  vs  
344 the  $R^2$  value for  $\text{Ca}^{2+}$  transient vs AP peak,  $n = 12$  fibers, paired t-test). The  $R^2$  value was larger  
345 for AP area vs  $\text{Ca}^{2+}$  transient because AP area closely mimicked the rapid decrease from 90% to  
346 10% of maximum that occurred for the  $\text{Ca}^{2+}$  transient (Fig 4D,  $p = 0.27$  vs the range of resting  
347 potentials for the decrease in  $\text{Ca}^{2+}$  transient, paired students t-test). These data suggest  
348 measurement of AP area is a significantly better predictor of failure of the  $\text{Ca}^{2+}$  transient than AP  
349 peak.



351

352 Fig 4- Correlation between  $\text{Ca}^{2+}$  transient and AP area. A) Shown in black are the AP traces and  
353  $\text{Ca}^{2+}$  transients recorded from 2 muscle fibers during infusion of 16 mM  $\text{K}^+$ . A horizontal line at  
354 -30 mV represents the cutoff for measurement of AP area and normalized AP peak. APs peaking  
355 below -30 mV had peaks and areas set to 0. Below the AP traces shown in blue are the  
356 corresponding  $\text{Ca}^{2+}$  transients. The stimulus artifact has been truncated in the AP traces for  
357 clarity. B) Plots of normalized AP peak, normalized AP area and normalized  $\text{Ca}^{2+}$  transient  
358 versus resting potential for 2 fibers. The numbers 1-5 on each plot represent the points  
359 corresponding to the 5 AP and  $\text{Ca}^{2+}$  transient traces shown in A. C) Plots of the normalized  $\text{Ca}^{2+}$   
360 transient versus either AP area or AP peak for the 2 fibers. The line of identity is drawn on each  
361 plot as a reference. The  $R^2$  value for each relationship is shown on the graph. D) Plots of the  
362 resting potential at which AP peak, AP area and the  $\text{Ca}^{2+}$  transients are 90% and 10% of maximal  
363 as well as the plot of the  $R^2$  values for the relationship between AP peak and AP area vs the  $\text{Ca}^{2+}$   
364 transient for each of the 12 fibers. The horizontal line in each plot represents the mean value for  
365 the data.

366

367

368 Discussion:

369 Prolonged intracellular recordings of individual muscle fibers during infusion of solution  
370 containing elevated  $\text{K}^+$  were performed while simultaneously imaging the  $\text{Ca}^{2+}$  transient  
371 triggered by action potentials (APs). The sudden failure of ECC with depolarization of the  
372 resting potential was not due to all-or-none failure of APs. An AP property that closely  
373 correlated with  $\text{Ca}^{2+}$  transient and thus the sudden failure of ECC was the integral of AP voltage  
374 with respect to time. Ours is the first study to determine quantitative relationships between  
375 resting potential, APs and the  $\text{Ca}^{2+}$  transients that trigger contraction during ECC in skeletal  
376 muscle. Understanding these relationships provides the foundation for future studies of  
377 depolarization-induced failure of ECC in various disease states such as periodic paralysis.

378

379 The integral of AP voltage with respect to time as the determinant of the  $\text{Ca}^{2+}$  transient

380 In this and a previous study, it was found that during elevation of extracellular  $\text{K}^+$ ,  
381 reduction in AP peaks occurs over a relatively wide range of resting potentials (Ammar et al.,

382 2015). The decrease is relatively modest between resting potentials of -85 to -65 mV, but  
383 becomes rapid between -65 mV and -55mV. This is due to the non-linear relationship between  
384 the density of Na<sup>+</sup> channels available to participate in APs and AP peak (Rich and Pinter, 2001,  
385 2003). The lack of all-or-none AP failure raised the question of why failure of muscle force  
386 generation is so sudden, occurring over a narrow range of resting potentials (Holmberg and  
387 Waldeck, 1980; Renaud and Light, 1992; Cairns et al., 1997; Yensen et al., 2002; Cairns et al.,  
388 2011; Pedersen et al., 2019). Ca<sup>2+</sup> imaging during depolarization revealed the answer: there is  
389 failure of the Ca<sup>2+</sup> transient over a narrow range of resting potentials.

390 Our study and previous studies suggest Ca<sup>2+</sup> release from the sarcoplasmic reticulum  
391 begins to be triggered at voltages of -30 to -20 mV and becomes maximal at voltages above +10  
392 mV (Wang et al., 1999; Braubach et al., 2014). Because APs peaking below -30 mV do not  
393 trigger elevation of Ca<sup>2+</sup>, we set -30 mV as 0 and normalized AP peaks. Despite normalization,  
394 the relationship between AP peak and Ca<sup>2+</sup> transient was not as close as we had hoped: the  
395 reduction in the AP peak still occurred more gradually than the drop in Ca<sup>2+</sup> transient. This  
396 caused us to look for a measure of APs that would more closely correlate with the Ca<sup>2+</sup> transient.

397 It has previously been reported that depolarization of the resting potential triggers  
398 widening of APs (Yensen et al., 2002). To incorporate consideration of both peak voltage and  
399 AP half width into our measure of APs, we took the integral of AP voltage with respect to time.  
400 This measure of APs closely correlated with the decrease in Ca<sup>2+</sup> transient responsible for failure  
401 of ECC secondary to depolarization of the resting potential. AP area did not correlate with the  
402 increase in Ca<sup>2+</sup> transient occurring with mild depolarization of the resting potential. There is  
403 still a factor yet to be determined that is responsible for the initial increase in Ca<sup>2+</sup> transient with  
404 depolarization of the resting potential.

405 The finding that AP integral more closely correlates with failure of the Ca<sup>2+</sup> transient than  
406 did AP peak makes biophysical sense. Depolarization during an AP triggers elevation of  
407 intracellular Ca<sup>2+</sup> by causing movement of gating charges in Cav1.1 channels in the t-tubules  
408 (Kovacs et al., 1979; Rios and Brum, 1987; Garcia et al., 1994). The movement of the voltage-  
409 sensing particles cause further conformational rearrangements within Cav1.1 which open RyR1  
410 and provide a conduit for release of Ca<sup>2+</sup> from the sarcoplasmic reticulum into the myoplasm.  
411 The total gating charge is dependent on both voltage and time (Schneider and Chandler, 1973).  
412 Thus, the longer the membrane potential remains depolarized during an AP, the greater the

413 gating charge movement produced by Cav1.1 channels until saturation is reached. Established  
414 data on Cav1.1 gating charge movement indicates that both the magnitude and time-course of  
415 gating charge movement in response to membrane potential changes are complex and non-linear  
416 (Gregorio et al., 2017). The release of  $\text{Ca}^{2+}$  in response to Cav1.1 gating charge movement is  
417 also non-linear. The use of the AP area metric as described in this work is therefore a  
418 simplification of the underlying processes.

419 There are several limitations of our study. One is that the  $\text{Ca}^{2+}$  indicator used is relatively  
420 high affinity (Chen et al., 2013; Shang et al., 2014; Dana et al., 2019), such that saturation of the  
421 dye may have caused us to underestimate the magnitude of the  $\text{Ca}^{2+}$  transient. One factor that  
422 might contribute to the sudden failure of the  $\text{Ca}^{2+}$  transient independent of AP area, is  
423 inactivation of  $\text{Ca}^{2+}$  release (Cota et al., 1984; Chua and Dulhunty, 1988; Schneider and Simon,  
424 1988; Robin and Allard, 2013; Gregorio et al., 2017; Hernandez-Ochoa and Schneider, 2018).  
425 The voltage dependence of Cav1.1 gating charge availability has been measured and found to  
426 have a midpoint of -57 mV (Gregorio et al., 2017); the same membrane potential at which we  
427 found the  $\text{Ca}^{2+}$  transient was half maximal. We did not separate the effect of depolarization of  
428 the resting potential on APs from the effect of depolarization of resting potential on  $\text{Ca}^{2+}$  release  
429 from the sarcoplasmic reticulum. Finally, we chose a voltage cut off for the integral of AP of -30  
430 mV. This may not be the correct cut off for all fibers. As shown in Fig 3F. there was a 15 mV  
431 range of AP peaks in different fibers at which there began to be a  $\text{Ca}^{2+}$  transient. Despite all the  
432 caveats, the close correlation between AP area and  $\text{Ca}^{2+}$  transient suggests the approach has  
433 value and may prove useful in future studies of ECC.

434

#### 435 The definition of an AP

436 Textbooks often describe APs as sudden depolarizations, which are all-or-none (Boron  
437 and Boulpaep, 2017; Purves et al., 2018). This definition derives from the finding that AP  
438 amplitude and conduction are independent of the amount of current injected (Cole and Curtis,  
439 1939). APs triggered at a normal muscle resting potential of -80 to -85 mV are all-or-none.  
440 However, with depolarization of the resting potential there is graded reduction of the AP peak  
441 such that AP amplitude ranges from 120 mV to below 10 mV (the current study and (Rich and  
442 Pinter, 2003; Quinonez et al., 2010; Ammar et al., 2015; Miranda et al., 2020; Uwera et al.,  
443 2020)).

444 If APs are not all-or-none, how does one define what is and what isn't an AP? One  
445 definition might be transient depolarizations that propagate the length of the fiber. However, to  
446 use this definition propagation of APs along the length of the fiber must be measured, which  
447 although possible, is not trivial (Riisager et al., 2014). As we did not study AP propagation, our  
448 study does not shed light on whether failure of AP propagation contributes to failure of ECC  
449 with depolarization of the resting potential.

450 A second definition of APs could be depolarizations that trigger elevation of intracellular  
451  $\text{Ca}^{2+}$ . The focus on elevation of  $\text{Ca}^{2+}$  is appealing since it is the link between APs and ECC.  
452 With this definition, the presence of an AP would always correlate with successful ECC.  
453 However, this definition requires concurrent imaging of intracellular  $\text{Ca}^{2+}$ , which limits  
454 applicability.

455 A recent study defined inexcitable fibers as fibers, “for which the membrane potential did  
456 not change at all following a stimulation” (Uwera et al., 2020). Although not explicitly stated, a  
457 corollary of this definition is that any depolarization occurring after termination of a stimulation,  
458 no matter how small, is an AP. With this definition, APs can vary widely in amplitude, ability to  
459 conduct along the length of the fiber, and ability to trigger ECC. The lack of functional  
460 correlation is a weakness, but this definition avoids making binary decisions about what is and  
461 what isn't an AP, which our study suggests would be difficult. Thus, despite its limitations, we  
462 favor this definition for studies of muscle diseases with failure of ECC caused by depolarization  
463 of the resting potential.

464

#### 465 Slow Inactivation of Na channels likely contributes to depolarization-induced failure of ECC

466 Recording in individual fibers during infusion of 16 mM  $\text{K}^+$  resulted in higher AP peaks  
467 at a given resting potential than were obtained from sampling fibers from muscles perfused with  
468 different concentrations of  $\text{K}^+$  (Fig 1D vs Fig 2D). One potential explanation of this difference is  
469 the speed of depolarization. In recordings from individual fibers, infusion of 16 mM  $\text{K}^+$   
470 triggered a ~30 mV depolarization over several minutes. When sampling fibers from muscles in  
471 solutions with varying  $\text{K}^+$  concentrations, muscles were incubated in each solution for 20  
472 minutes prior to recording of APs. The prolonged depolarization allows for greater slow  
473 inactivation of  $\text{Na}^+$  channels, (Ruff, 1999; Rich and Pinter, 2003), such that AP peak decreased  
474 at more negative resting potentials. In disease states causing depolarization of the resting

475 potential, depolarization is generally prolonged such that slow inactivation of Na<sup>+</sup> channels  
476 would play an important role in the reduction of AP peak.

477

478 Summary

479 Ours is the first study to establish quantitative relationships between resting potential,  
480 APs and generation of the Ca<sup>2+</sup> transient in individual fibers. Understanding these relationships  
481 provides the foundation for studies of depolarization-induced failure of ECC in a variety of  
482 muscle diseases.

483

484

485

486 References:

487

488 Adrian RH, Costantin LL, Peachey LD (1969) Radial spread of contraction in frog muscle fibres.

489 J Physiol 204:231-257.

490 Allen DG, Lamb GD, Westerblad H (2008) Skeletal muscle fatigue: cellular mechanisms.

491 Physiol Rev 88:287-332.

492 Ammar T, Lin W, Higgins A, Hayward LJ, Renaud JM (2015) Understanding the physiology of

493 the asymptomatic diaphragm of the M1592V hyperkalemic periodic paralysis mouse. J

494 Gen Physiol 146:509-525.

495 Bannister RA, Beam KG (2013) Ca(V)1.1: The atypical prototypical voltage-gated Ca(2)(+)

496 channel. Biochim Biophys Acta 1828:1587-1597.

497 Boron WF, Boulpaep EL (2017) Medical Physiology, Third Edition. Philadelphia, PA: Elsevier.

498 Braubach P, Orynbayev M, Andronache Z, Hering T, Landwehrmeyer GB, Lindenberg KS,

499 Melzer W (2014) Altered Ca(2+) signaling in skeletal muscle fibers of the R6/2 mouse, a

500 model of Huntington's disease. J Gen Physiol 144:393-413.

501 Cairns SP, Leader JP, Loiselle DS (2011) Exacerbated potassium-induced paralysis of mouse

502 soleus muscle at 37 degrees C vis-a-vis 25 degrees C: implications for fatigue. K+ -

503 induced paralysis at 37 degrees C. Pflugers Arch 461:469-479.

504 Cairns SP, Buller SJ, Loiselle DS, Renaud JM (2003) Changes of action potentials and force at

505 lowered [Na+]o in mouse skeletal muscle: implications for fatigue. Am J Physiol Cell

506 Physiol 285:C1131-1141.

507 Cairns SP, Hing WA, Slack JR, Mills RG, Loiselle DS (1997) Different effects of raised [K+]o

508 on membrane potential and contraction in mouse fast- and slow-twitch muscle. Am J

509 Physiol 273:C598-611.

510 Cannon SC (2015) Channelopathies of skeletal muscle excitability. Compr Physiol 5:761-790.

511 Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA,

512 Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS (2013) Ultrasensitive

513 fluorescent proteins for imaging neuronal activity. Nature 499:295-300.

514 Chua M, Dulhunty AF (1988) Inactivation of excitation-contraction coupling in rat extensor

515 digitorum longus and soleus muscles. J Gen Physiol 91:737-757.



- 516 Cole KS, Curtis HJ (1939) Electric Impedance of the Squid Giant Axon during Activity. *J Gen*  
517 *Physiol* 22:649-670.
- 518 Cota G, Nicola Siri L, Stefani E (1984) Calcium channel inactivation in frog (*Rana pipiens* and  
519 *Rana moctezuma*) skeletal muscle fibres. *J Physiol* 354:99-108.
- 520 Dana H, Sun Y, Mohar B, Hulse BK, Kerlin AM, Hasseman JP, Tsegaye G, Tsang A, Wong A,  
521 Patel R, Macklin JJ, Chen Y, Konnerth A, Jayaraman V, Looger LL, Schreiter ER,  
522 Svoboda K, Kim DS (2019) High-performance calcium sensors for imaging activity in  
523 neuronal populations and microcompartments. *Nat Methods* 16:649-657.
- 524 Delbono O, Kotsias BA (1987) Relation between action potential duration and mechanical  
525 activity on rat diaphragm fibers. Effects of 3,4-diaminopyridine and tetraethylammonium.  
526 *Pflugers Arch* 410:394-400.
- 527 Dulhunty AF (2006) Excitation-contraction coupling from the 1950s into the new millennium.  
528 *Clin Exp Pharmacol Physiol* 33:763-772.
- 529 Friedrich O, Reid MB, Van den Berghe G, Vanhorebeek I, Hermans G, Rich MM, Larsson L  
530 (2015) The Sick and the Weak: Neuropathies/Myopathies in the Critically Ill. *Physiol*  
531 *Rev* 95:1025-1109.
- 532 Garcia J, Tanabe T, Beam KG (1994) Relationship of calcium transients to calcium currents and  
533 charge movements in myotubes expressing skeletal and cardiac dihydropyridine  
534 receptors. *J Gen Physiol* 103:125-147.
- 535 Gong B, Legault D, Miki T, Seino S, Renaud JM (2003) KATP channels depress force by  
536 reducing action potential amplitude in mouse EDL and soleus muscle. *Am J Physiol Cell*  
537 *Physiol* 285:C1464-1474.
- 538 Gregorio JF, Pequera G, Manno C, Rios E, Brum G (2017) The voltage sensor of excitation-  
539 contraction coupling in mammals: Inactivation and interaction with Ca<sup>2+</sup>. *Journal of*  
540 *General Physiology* 149:1041-1058.
- 541 Hernandez-Ochoa EO, Schneider MF (2018) Voltage sensing mechanism in skeletal muscle  
542 excitation-contraction coupling: coming of age or midlife crisis? *Skelet Muscle* 8:22.
- 543 Holmberg E, Waldeck B (1980) On the possible role of potassium ions in the action of  
544 terbutaline on skeletal muscle contractions. *Acta Pharmacol Toxicol (Copenh)* 46:141-  
545 149.

- 546 Kovacs L, Rios E, Schneider MF (1979) Calcium transients and intramembrane charge  
547 movement in skeletal muscle fibres. *Nature* 279:391-396.
- 548 Lehmann-Horn F, Jurkat-Rott K, Rudel R (2008) Diagnostics and therapy of muscle  
549 channelopathies--Guidelines of the Ulm Muscle Centre. *Acta Myol* 27:98-113.
- 550 Melzer W, Herrmann-Frank A, Luttgau HC (1995) The role of Ca<sup>2+</sup> ions in excitation-  
551 contraction coupling of skeletal muscle fibres. *Biochim Biophys Acta* 1241:59-116.
- 552 Miranda DR, Reed E, Jama A, Bottomley M, Ren H, Rich MM, Voss AA (2020) Mechanisms of  
553 altered skeletal muscle action potentials in the R6/2 mouse model of Huntington's  
554 disease. *Am J Physiol Cell Physiol*.
- 555 Overgaard K, Nielsen OB (2001) Activity-induced recovery of excitability in K(+)-depressed rat  
556 soleus muscle. *Am J Physiol Regul Integr Comp Physiol* 280:R48-55.
- 557 Overgaard K, Nielsen OB, Flatman JA, Clausen T (1999) Relations between excitability and  
558 contractility in rat soleus muscle: role of the Na<sup>+</sup>-K<sup>+</sup> pump and Na<sup>+</sup>/K<sup>+</sup> gradients. *J*  
559 *Physiol* 518:215-225.
- 560 Pedersen KK, Cheng AJ, Westerblad H, Olesen JH, Overgaard K (2019) Moderately elevated  
561 extracellular [K(+)] potentiates submaximal force and power in skeletal muscle via  
562 increased [Ca(2+)]<sub>i</sub> during contractions. *Am J Physiol Cell Physiol* 317:C900-C909.
- 563 Pedersen TH, Clausen T, Nielsen OB (2003) Loss of force induced by high extracellular [K<sup>+</sup>] in  
564 rat muscle: effect of temperature, lactic acid and beta2-agonist. *J Physiol* 551:277-286.
- 565 Pratt FH (1917) The all-or-none principle in graded response of muscle contraction. *American*  
566 *Journal of Physiology* 44:517-542.
- 567 Purves D, Augustine GJ, Fitzpatrick D, Hall WC, S. LA, Mooney RD, Platt ML, White L (2018)  
568 *Neuroscience*. New York, New York: Oxford University Press.
- 569 Quinonez M, Gonzalez F, Morgado-Valle C, DiFranco M (2010) Effects of membrane  
570 depolarization and changes in extracellular [K(+)] on the Ca (2+) transients of fast  
571 skeletal muscle fibers. Implications for muscle fatigue. *J Muscle Res Cell Motil* 31:13-  
572 33.
- 573 Renaud JM, Light P (1992) Effects of K<sup>+</sup> on the twitch and tetanic contraction in the sartorius  
574 muscle of the frog, *Rana pipiens*. Implication for fatigue in vivo. *Can J Physiol*  
575 *Pharmacol* 70:1236-1246.

576 Rich MM, Pinter MJ (2001) Sodium channel inactivation in an animal model of acute  
577 quadriplegic myopathy. *Annals of Neurology* 50:26-33.

578 Rich MM, Pinter MJ (2003) Crucial role of sodium channel fast inactivation in muscle fibre  
579 inexcitability in a rat model of critical illness myopathy. *J Physiol* 547:555-566.

580 Riisager A, Duehmke R, Nielsen OB, Huang CL, Pedersen TH (2014) Determination of cable  
581 parameters in skeletal muscle fibres during repetitive firing of action potentials. *J Physiol*  
582 592:4417-4429.

583 Rios E, Brum G (1987) Involvement of dihydropyridine receptors in excitation-contraction  
584 coupling in skeletal muscle. *Nature* 325:717-720.

585 Robin G, Allard B (2013) Major contribution of sarcoplasmic reticulum Ca(2+) depletion during  
586 long-lasting activation of skeletal muscle. *J Gen Physiol* 141:557-565.

587 Ruff RL (1999) Effects of temperature on slow and fast inactivation of rat skeletal muscle Na(+)  
588 channels. *Am J Physiol* 277:C937-947.

589 Schneider MF, Chandler WK (1973) Voltage dependent charge movement of skeletal muscle: a  
590 possible step in excitation-contraction coupling. *Nature* 242:244-246.

591 Schneider MF, Simon BJ (1988) Inactivation of calcium release from the sarcoplasmic reticulum  
592 in frog skeletal muscle. *J Physiol* 405:727-745.

593 Shang W, Lu F, Sun T, Xu J, Li LL, Wang Y, Wang G, Chen L, Wang X, Cannell MB, Wang  
594 SQ, Cheng H (2014) Imaging Ca<sup>2+</sup> nanosparks in heart with a new targeted biosensor.  
595 *Circ Res* 114:412-420.

596 Uwera F, Ammar T, McRae C, Hayward LJ, Renaud JM (2020) Lower Ca<sup>2+</sup> enhances the K<sup>+</sup>-  
597 induced force depression in normal and HyperKPP mouse muscles. *J Gen Physiol* 152.

598 van Lunteren E, Pollarine J, Moyer M (2006) Inotropic effects of the K<sup>+</sup> channel blocker 3,4-  
599 diaminopyridine: differential responses of rat soleus and extensor digitorum longus. *IEEE*  
600 *Trans Neural Syst Rehabil Eng* 14:419-426.

601 Wang ZM, Messi ML, Delbono O (1999) Patch-clamp recording of charge movement, Ca<sup>2+</sup>  
602 current, and Ca<sup>2+</sup> transients in adult skeletal muscle fibers. *Biophys J* 77:2709-2716.

603 Yensen C, Matar W, Renaud JM (2002) K<sup>+</sup>-induced twitch potentiation is not due to longer  
604 action potential. *Am J Physiol Cell Physiol* 283:C169-177.

605