Donnan dominated ion homeostasis and the longevity of ischemic Na⁺-loaded dystrophic skeletal muscle

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ABSTRACT

The inherited muscle-wasting disease, Duchenne muscular dystrophy (DMD), renders skeletal muscle fibers (SMFs) Na^+ -overloaded, ischemic, membrane-damaged, cation-leaky, depolarized, and prone to myogenic firing. DMD fibers nevertheless survive up to 3 decades before succumbing to Ca^{2+} -necrosis. The Ca^{2+} -necrosis is explicable, the longevity is not. Modeling here shows that SMFs' ion homeostasis strategy, a low-cost resilient Pump-Leak/Donnan feedback process we term "Donnan dominated", underpins that longevity. Together, SMFs' huge chloride-permeability and tiny sodium-permeability minimize excitability and pump costs, facilitating the outsized SMF pump-reserve that lets DMD fibers withstand deep ischemia and leaky channels. We illustrate how, as these impairments intensify, patients' chronic Na^+ -overload (now non-invasively evident via Na^{23} -MRI) would change. In simulations, prolonged excitation ($\rightarrow$ physiological Na^+ -overloading) and/or intense ischemia ($\rightarrow$ too little Na^+ -pumping) and accumulated bleb-damage ($\rightarrow$ too much Na^+ -leaking) eventually trigger Ca^{2+} -overloading conditions. Our analysis implies an urgent need to identify SMFs' pivotal small P_{Na} , thereby opening new therapeutic remediation routes.

INTRODUCTION

Skeletal muscle fibers (SMFs) and dystrophin

SMFs are large long-lived multinucleate force-generating cells that are also subject to mechanical stresses. Their sarcolemmal bilayer, as for any plasma membrane, represents the fragile ~4 nm wide osmotic and electrical boundary across which electrical signals and ion homeostasis are mediated. In SMFs, bilayer tearing and bleb-damage (dissociation of bilayer from adherent cytoskeleton proteins) is minimized by dystrophin, an exceptionally long filamentous protein bound to actin, microtubules, lipids and more, and anchored at the massive transbilayer sarcolemmal dystroglycan complex (Campbell and Kahl 1989; Ibraghimov-Beskrovnya et al 1992; Prins et al 2009; Khairallah et al 2012; Sarkis et al 2013; Belanto et al 2014; Dos Santos Morais et al 2018). Dystrophin acts as an entropic spring shock absorber (Le et al 2018), providing mechanical resilience while rendering the membrane skeleton "smart" through its interactions with various signaling proteins (Constantin 2014; Allen et al 2016). Sporadically, to compensate for accumulated wear and tear, adult SMFs regenerate; this is achieved by SMF fusion with stem (satellite) cells that have had a surge of dystrophin expression (Wang et al 2013; Dumont et al 2015; Chang et al 2016).

In the X-linked muscle-wasting disease, Duchenne muscular dystrophy (DMD), patients become non-ambulatory in their teens and live <3 decades dealing with wide-ranging ion homeostatic/metabolic impairment, most notably muscle ischemia (Allen et al 2016; Bishop et al 2018; Huong et al 2018; Gerhalter et al 2019). The chronic Na^+ -overload of dystrophic SM was first reported in 1957 (**Table 1**, item 1). In 1988, Zubrzycka-Gaarn and colleagues reported that, unexpectedly, the DMD gene encodes not an "energy" enzyme but dystrophin, a membrane skeletal protein (Allen et al 2016), and dystrophin-minus mice, *mdx* (Ryder-Cook et al 1988), were introduced.

Mechanistic clarity on how DMD's lack of dystrophin engenders persistent skeletal muscle ischemia is still a work-in-progress (e.g., Timpani et al 2015; Moore et al 2020) but the ischemia has multiple facets (**Figure 1A,B,C**). Early *mdx* SMF membrane studies showed that expression of Na^+/K^+ -ATPase is not impaired, with both Anderson (1991) and Dunn et al (1995) reporting pump-protein at above-normal levels. Anderson's work did, however, include findings consistent with "pump-strength" sub-normality, the supra-normal pump-protein-density notwithstanding; *mdx* sarcolemma has abnormal phospholipid profiles, consistent with the cumulative *mdx* bleb-damage from mechanical strain, reactive oxygen species and $\uparrow\text{Ca}^{2+}$ (Petrof et al 1993; Dudley et al 2016) and consistent with pump dysregulation (see Hossain and Clarke 2019).

During muscle exertion, dystrophin-dependent signaling normally ensures adequate microcirculation as follows: nNOS μ , a nitric oxide(NO)-synthetase bound to the dystrophin-dystroglycan complex, responds to exertion by locally increasing vasodilation-inducing NO (Sander et al 2000; Allen et al 2016). Lacking dystrophin to position nNOS μ , DMD fibers experience “functional ischemia” (Asai et al 2007; Thomas 2013; Altamirano et al 2014; Nelson et al 2015; Rebolledo et al 2016; Percival 2018) so contractile activity dangerously exacerbates DMD fibers’ persistent ischemia. Though clinically, “ischemia” means inadequate blood-flow, for modeling here it will signify sub-normal (<100%) Na⁺/K⁺-ATPase **strength** from any cause.

The DMD/*mdx* sarcolemma is mechanically vulnerable not only to bleb-damage, but to bilayer micro-tears and rupture (**Figure 1B,C**) (Petrof et al 1993, 2002; Menke and Jockusch 1991,1995; Dudley et al 2006; García-Pelagio et al 2011; Allen et al 2016; Houang et al 2018). Overexpression of the dystrophin-related protein, utrophin, substitutes only partially (e.g. Belanto et al 2014). Though micro-tears occur easily in DMD/*mdx*, fast exocytosis-based repair plus endocytic retrieval of resealed-patches functions normally (McNeil and Steinhardt 2003; Hody et al 2019; Barthélémy et al 2018; Corrotte et al 2013; Andrews et al 2014). Though myogenic contractures are extra-perilous for *mdx* fibers (Claflin and Brooks 2008) and though fiber-stretch injury can leave *mdx* fibers depolarized and inexcitable, recovery occurs within days or weeks (Anderson 1991; Call et al 2013; Pratt et al 2015; Baumann et al 2020). The fragility of DMD/*mdx* fibers is manifest here, along with the remarkable resilience of SMF ion homeostasis.

Ion homeostasis and DMD SMFs

Of the many muscular dystrophies, DMD is not only the commonest, but the most severe (Datta and Ghosh 2020). Nevertheless, DMD fibers can survive 20-30 years, progressively more debilitated by chronic ischemia, plagued by sarcolemmal damage. Though sarcolemmal tear-repair is a must, defective-repair myopathies (dysferlin muscular dystrophies) are less severe than DMD (Bansal et al 2003; Bittel et al 2020). What pre-disposes even DMD-stricken SMFs for longevity, we posit, is their evolutionarily-ancient energy-efficient ion homeostatic strategy.

As **Figure 8** and its legend summarize, a companion paper (Joos & Morris submitted) explains the distinctive Donnan dominated strategy adopted by SMFs, contrasting it with the better-known (Dijkstra et al 2016) Pump-leak dominated strategy of neurons. SMFs are low duty-cycle excitable cells (i.e., mostly at V_{rest}) whose inexpensive steady-state is achieved by exploiting impermeant myoplasmic anions (Donnan effectors) in passive collaboration with [big P_{Cl}] (ClC-1-based). This stabilizes a V_{rest} that, thanks to exceedingly [small I_{NaLeak}], is deeply hyperpolarized. The [small I_{NaLeak}] simultaneously fosters large pump-reserves that ensure resumption of steady-state even after abnormally large perturbations.

Here our aim is: **1)** to detail, with reference to the dystrophin-minus deficits of DMD-SMFs (ischemia and sarcolemmal damage), how their Donnan dominated P-L/D strategy safeguards such fibers, and **2)** to explore how leaky Na⁺-permeant channels (in damaged sarcolemma) would contribute to the chronic Na⁺-overload, myogenic contractures and eventual susceptibility to Ca²⁺-necrosis that besets DMD fibers.

When pump-strength falls so low that the P-L/D feedbacks fail, Donnan forces become lethal through irreversible osmotic (“cytotoxic”) swelling. This is how neurons die in cortical spreading depression (Rungta et al 2015; Dijkstra et al 2016; Dreier et al 2018) but for DMD fibers, Ca²⁺-necrotic processes typically intervene (Burr & Molkentin 2015; Timpani et al 2015; Murphy et al 2019). In DMD fibers, cause-of-death assignment is complicated by ischemia impacting both Ca²⁺ (Smith et al 2013) and ion (Na⁺/K⁺) homeostasis. With SMF ion homeostasis not widely understood even for healthy fibers, attention has mostly focused on proximate causes of DMD fiber-loss monitored via Ca²⁺-dyes, as when fiber-stretch or repeated myogenic action potentials (APs) trigger catastrophic Ca²⁺ influx and

contractures (Yeung et al 2005; Claflin & Brooks 2008). Therapeutically it would be preferable, however, to intervene, where possible, upstream of lethal Ca^{2+} -dysregulation.

Like healthy SMFs, DMD fibers rely on E_{Na} for APs and to fuel Na^{+} -transporters (glucose, pH, multiple ions, Ca^{2+} , etc (Usher-Smith et al 2009)). The chronic Na^{+} -overload of DMD fibers (**Table 1**) is, by definition, non-lethal (Weber et al 2011,2012). That being said, when reverse operation of $\text{Na}^{+}/\text{Ca}^{2+}$ -exchangers causes *mdx* fiber death, diminished E_{Na} is a pre-requisite (Burr et al 2014). Here again, what triggers Ca^{2+} -necrosis is severe upstream Na^{+} -overload. Though Ca^{2+} -overload typically proves lethal sooner than cytotoxic swelling, fixing Na^{+} homeostasis could be most fruitful therapeutically.

Reviewing the consensus view (Allen et al 2016) that Ca^{2+} -necrosis underlies DMD fiber loss, Burr and Molkenkin (2015) write that “given the known mechanical defects within the dystrophic plasma membrane...alterations in calcium and sodium levels likely stem...from excessive activation of various channels and exchangers”. Implicit, by elimination, is the assumption that “too much Na^{+} -leak” across bleb-damaged sarcolemma explains DMD Na^{+} -overload. Our P-L/D analysis indicates, however, that “too little Na^{+} -pumping” is also critical for DMD Na^{+} -overload.

Addressing the basis of chronic Na^{+} -overload via P-L/D modeling

For almost 70 years ion homeostatic dysfunction has been recognized as a DMD trait, but this has yet to be addressed through a theoretical framework. Chronic Na^{+} -overload was first reported for dystrophic muscle in the 1950s and is presently monitored non-invasively in patients via ^{23}Na -MRI (**Table 1**, items **5,9,10**). Ion homeostatic impairment of V_m regulation presents clinically as myogenic contractility (Ishpekova et al 1999; Nojszewska et al 2017). Muscle tissue edema (versus SMF edema) in DMD has been confusing but concurrent ^{23}Na -MRI and proton-MRI (to gauge fiber- H_2O) (Weber et al 2011; Gerhalter et al 2017,2019) is providing greater clarity. Quantifying muscle ischemia has been problematic, but that is changing (Zhang et al 2020; Deitz et al 2020)).

Our theoretical framework is the ultra-simple P-L/D excitable SMF model, SM-CD (Skeletal Muscle Charge Difference; Joos & Morris submitted), with specified modifications to mimic the DMD condition. Two classes of “leaky” Na^{+} -permeant channels expected in damaged sarcolemma are tested and DMD chronic ischemia is ascribed, partly, to non-functional pump-protein in damaged sarcolemma (**Figure 1A**). We show both how ischemic membrane-damaged DMD fibers could remain viable, and how their typical demise (via Ca^{2+} -necrosis) relates to treacherous P-L/D thresholds. In compartment syndrome, similar issues arise, albeit far more rapidly than for DMD. Commonalities include profound ischemia, muscle damage and the imperative to avoid irreversible thresholds (Johnstone & Ball 2019; Tatman et al 2020). Dooley and Chiasson (2014; see too, Siegel 1992) argue, interestingly, that DMD patients’ sustained limb contractures can secondarily produce (fasciotomy-relieved) compartment syndrome.

Cell and molecular therapies are emerging for DMD (e.g. Datta et al 2020), concurrent with improved non-invasive monitoring of patients’ ion homeostatic indicators (Mankodi et al 2017; Bishop et al 2018; Gerhalter et al 2019; Ropars et al 2020; Meng et al 2020; Pennati et al 2020). Whether Na^{+} -overload in DMD patients indicates fiber swelling that “is likely osmotically relevant” and thus “may contribute to the progressive muscle degeneration” (Weber et al 2011,2012) has been unclear. While recent patient measurements confirm the Na^{+} -overload (Gerhalter et al 2017, 2019) they find it to be mostly non-osmotic. Our P-L/D -based analysis predicts this. As ischemia deepens, it shows, SMF Na^{+} -overload would remain essentially non-osmotic till the attendant ischemic depolarization triggered myogenic firing. P-L/D analysis explains both tolerance of anoxic episodes and the limits to that tolerance, showing how over-stimulation plus membrane damage become fiber-lethal.

Our findings constitute a low-resolution conceptual map for the major contours of DMD’s ion homeostatic impairment. In future, SM-CD could be augmented with Na^{+} -transporters, Nav1.4’s slow-gating kinetics, pump characteristics specific to SMFs, and more, to enhance its sophistication for

biomedical research. **To recap: 1)** DMD fibers are chronically ischemic, Na^+ -loaded, leaky to Na^+ and overly-contractile. **2)** DMD fibers can survive for decades. **3)** Non-invasive monitoring of patients' ion homeostatic indicators is improving sharply just as potential therapies are coming on-line. **4)** SMFs use a $[\text{big } P_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$ or Donnan dominated strategy for ion homeostasis and an accessible theoretical framework is now available. **5)** We show how robust ion homeostasis safeguards SMFs during deepening ischemia and/or worsening Na^+ -leaks; their $[\text{big } P_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$ strategy, we argue, substantially explains DMD fibers' remarkable longevity.

RESULTS

Overview

SM-CD, a $[\text{big } P_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$ ion homeostasis (P-L/D) model for excitable SMFs is used to address the issue of DMD longevity. SMF's $[\text{big } P_{\text{Cl}}]$ is mostly its well-studied (SMF-specific) channel, ClC-1 (Pedersen et al 2016; Park and Mackinnon 2018; Wang et al 2019). Curiously, though ClC-1 's role in passively stabilizing SMF's hyperpolarized V_{rest} is well understood, its role in SMF ion homeostasis *per se* goes unrecognized (Jentsch and Pusch 2018; Jeng et al 2020). A recurring theme in the Results is the pivotal contribution of $[\text{small } I_{\text{Naleak}}]$. The small P_{Na} through which $[\text{small } I_{\text{Naleak}}]$ flows is unidentified.

Since DMD fibers are chronically ischemic, we first itemize benefits that ischemic SM-CD gleans from the P_{Na} and P_{Cl} components of " $[\text{big } P_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$ ". The full excitable SM-CD ensemble -- P_{Na} , P_{K} , P_{Cl} , pump, H_2O , Donnan effectors, Nav, Kv, C_m -- is then examined in contexts relevant to DMD (bouts of anoxia/recovery, ischemia at varying intensity, damage-induced Na^+ -leaks through cation and Nav channels, and over-stimulation). Globally this reveals: extreme insult/injury is needed to overwhelm the Donnan dominated P-L/D system. Demise occurs when, Na^+ -influx finally exceeds the pump's capacity (pump-reserve), eliciting myogenic APs and cytotoxic swelling, i.e., *in silico* death by $[\text{Na}^+ + \text{Cl}^- + \text{H}_2\text{O}]$ -overload. *In situ*, a Ca^{2+} -necrosis *coup de grce* would likely supersede irreversible swelling. Because ^{23}Na -proton-MRI is being used to monitor DMD patients' ion homeostatic indicators, the last figure shows that myoplasmic Na^+ -overloads deemed "safe" 1) are "non-osmotic" but 2) vary substantially with membrane-damage and ischemic intensity.

Syncytial SMFs are even more efficient than depicted by SM-CD

For physiological perspective, comparisons are made in some instances between ion fluxes across the $2000 \mu\text{m}^2$ of SM-CD and CN-CD (CN-CD=Cortical Neuron Charge Density; initial Vol_{cell} and pump strengths are also identical, see **Table 2**).

SM-CD P-L/D resilience substantially exceeds that of CN-CD, a differential even more impressive if SMFs' distinctive syncytial morphology is factored in. Joos and Morris (submitted) estimate that, *in situ*, $2000 \mu\text{m}^2$ would service a myoplasmic volume ~10-15X that of SM-CD. Steady-state P-L/D values depend only on surface area, syncytial morphology therefore magnifies muscle tissue's resting efficiency. SMFs' rates of change (e.g., during rundown and recovery) would be slower than depicted by SM-CD, but for rundown, this is a boon, while for recovery, given SMFs' large pump-reserve and low duty-cycle, it would generally be unproblematic.

Small P_{Na} , pump-reserve, *mdx* fibers, a chronically ischemic fiber model

Hyperpolarizing I_{pump} counter-balances depolarizing I_{Naleak} across P_{Na} in P-L/D systems; maximal hyperpolarizing I_{pump} in SM-CD and CN-CD are identical. Though its $P_{\text{K}}:P_{\text{Na}}$ ratio makes resting SM-CD ~20 mV more negative than resting CN-CD, $[\text{small } I_{\text{Naleak}}]$ makes SM-CD's ATP-consumption 5.3X lower (its pump-reserve is thus 5.3X larger; pump-reserve is maximal/resting ATP-consumption or its equivalent, maximal/resting $I_{\text{pump}})$ (**Table 2**). Small P_{Na} is thus pivotal for SMFs' extreme ion homeostatic efficiency and

for their pump-reserve based robustness. Pump-reserve is 11-fold in SM-CD, while for rat muscle, 7-fold to 22-fold is reported (see Clausen 2013, 2015).

Pump-protein density in isolated *mdx* sarcolemma (Anderson 1981; Dunn 1993) exceeds that of control mice. Nevertheless, we assume that membrane-damage etc (**Figure 1A**) puts *operational mdx* pump-strength in the “ischemic” range (<100% of normal). In *mdx* fibers NF-κB-dependent modulators depress pumping and increase the Na⁺-overload of (**Table 1** item **4**; Miles et al 2011). In *mdx* fibers, stimulating NO pathways almost fully abolishes the Na⁺-overload of (Altamirano et al 2014; **Table 1**, item **7**); whether (as presumed) this is attributable to “more Na⁺-pumping” was not determined. In a rat diaphragm DMD-model (Lehmann-Horn et al 2012), eplerenone up-regulation of the Na/K-ATPase (via Tyr10 dephosphorylation of its α-subunit) results in ouabain-sensitive fiber repolarization (Breitenbach et al 2016). Already prescribed for DMD cardiomyopathy (Raman et al 2017), eplerenone has been considered for DMD skeletal muscle (pilot study; Glemser et al 2017; **Table 1**, items **5,6,9**).

While enabling rapid post-perturbation return to steady-state, large pump-reserves also provide generous capacity for E_{Na}-exploiting Na⁺-transporters. Since SM-CD lacks Na⁺-transporters, its steady-state [Na⁺]_i (3.7 mM) is understandably lower than in healthy mouse SMFs (**Table 1** items **2,4,7,8**; i.e., 5.3-13 mM). Including transporters would complicate attempt to assess whether chronic Na⁺-overload could develop solely from: **1**) too little Na⁺-pumping (ischemia) or **2**) too much Na⁺-leaking (leaky channels). Using *mdx*-variants, Burr et al (2014), showed that reverse operation of Na⁺/Ca²⁺-exchangers can trigger Ca²⁺-necrosis in SMFs, provided there is a pre-existing Na⁺-overload. To design therapeutic approaches, mechanistic clarity on the provenance of Na⁺-overload is needed.

To represent deeply ischemic viable DMD fibers, LA-CD (30% pump-strength of SM-CD, pump-reserve=3.4-fold, (**Table 2**)) is used. Its 6.5 mM [Na⁺]_i is still within the range reported for healthy mice but if, as in working mouse fibers, Na⁺-transporters (e.g., Na⁺/H⁺ exchanger; Iwata et al 2007) were depleting E_{Na}, [Na⁺]_i could easily rise into the (chronically Na⁺-overloaded) range reported for *mdx* fibers by Burr et al (2014) (**Table 1**, item **8**; 7.3 mM).

Even ignoring that healthy rodent SMF pump-reserves far exceed the 3.4-fold of LA-CD, its ion homeostasis is sub-par based on this: to maintain [Na⁺]_i=6.5 mM, LA-CD consumes ATP at 50.7 amol/s, whereas SM-CD, consuming essentially the same amount (51.1 amol/s), achieves [Na⁺]_i=3.7 mM (see **Figure 9 Bii**).

Small input impedance and [big P_{Cl}] in ischemic SM-CD

Small P_{Na} is imperative for the low-cost steady-state that lets chronically ischemic DMD fibers survive. For strongly hyperpolarized SMFs, [small I_{Naleak}] through small P_{Na} is workable because, to keep their excitability suitably low, SMFs use *not* big P_K, but [big P_{Cl}]. WD-CD, a counterfactual Pump-Leak dominated analog of SM-CD (same low input impedance, same V_{rest} but “big P_K, big P_{Na}, small P_{Cl}”; **Table 2**) shows this quantitatively (Joos & Morris, submitted).

In *mdx* (Cozzoli et al 2014) as in healthy fibers open CIC-1 channels dominate [big P_{Cl}]. Unwanted firing is detrimental to ischemic DMD fibers. To suppress such firing without metabolic penalty would be attractive. Excitability modulation via ΔP_{Cl} (Pedersen et al 2011, 2016) therefor reveals an ancillary benefit of the Donnan dominated strategy: in SM-CD, changing P_{Cl} say, ±10-fold changes neither steady-state ATP-consumption (51.1 amol/s) nor V_{rest} (-86.0 mV). Likewise for LA-CD. Electrophysiologically, this accords, e.g., with the finding that SMFs from wild-type and CIC-1-gene-null mice have the same V_{rest} (Novak et al 2015). Further, Bækgaard Nielsen et al (2017) find that, with heavy exercise, fast-twitch fibers initially inhibit CIC-1s but as stimulation continues, inhibition can suddenly reverse to dramatically increased CIC-1-opening, abolishing excitability.

The SMF/neuron contrast is especially stark here: whereas $\uparrow P_{Cl}$ hastens cytotoxic swelling in ischemic neurons (Rungta et al 2015; Dijkstra et al 2016; Joos and Morris submitted), ischemic SMFs deploy $\uparrow P_{Cl}$ for enhanced safety.

Small P_{Na} : anoxic rundown and recovery in SM-CD and LA-CD

Transient functional ischemia imperils DMD fibers. In **Figure 2Ai**, with $I_{pump}=0$, anoxic rundown of SM-CD to Donnan Equilibrium (DE) is slow (as per Joos & Morris submitted) but here, in addition, we examine pump-strength restoration (to 100%). Restoring pumps during rundown or even during ectopic firing (**Figure 2Aii,iii**) lets the system return quickly to steady-state; $[Na^+]_i$ -sensitive I_{pump} hyperpolarizes V_m until $[Na^+]_i$ renormalizes. Critically, however, once ectopic firing stops, pump-restoration no longer supports recovery (**Figure 2Aiv**); degradation continues, not to Donnan Equilibrium (DE), but to towards a pathological steady-state, DE-like, though ATP-consuming.

Nevertheless, even with pumps-off, note that the “[big P_{Cl}][small I_{NaLeak}]” “architecture” of SMF ion homeostasis offers exceptional protection in the form of a prolonged period of reversible rundown (issues relating to ischemia-reperfusion injury (Dudley et al 2006; Schmucker et al 2015; Li et al 2020) are beyond our scope). The [small I_{NaLeak}] is rate-limiting and keeps fully-anoxic SM-CD safe for 22.7 minutes. During the rundown, E_{Na} and E_K depletion is almost matched, and the extent of swelling is modest. Given the SA/V ratios of actual myofibers (as explained above) their “safe” anoxic-rundown durations would substantially exceed SM-CD’s.

Figure 2Bi shows that a slight reduction of already-small P_{Na} markedly increases its benefits. For SM-CD, $V_{rest}=-86$ mV. When P_{Na} drops from $0.3 \mu m^3/s \rightarrow 0.22 \mu m^3/s$, $V_{rest}=-90$ mV and ATP-consumption falls ($51.1 \text{ amol/s} \rightarrow 38.7 \text{ amol/s}$), boosting pump-reserve from 11.1-fold $\rightarrow$ 14.6-fold. With P_{Na} reduced, anoxic rundown slows: ectopic firing begins at 34 not 22.7 minutes (**2Bii,iii** expands the start and termination of firing) while recovery initiated during rundown (not shown) is quicker.

The primacy of small P_{Na} is made further evident by the fact that anoxic rundown speed for SM-CD is almost indifferent to P_{Cl} : SM-CD “goes ectopic” at 20.7 minutes for $0.1XP_{Cl}$, and at 23.8 minutes for $10XP_{Cl}$ (not shown). Thus, while $\uparrow P_{Cl}$ could abruptly $\downarrow$ endplate-triggered excitability under energy-depleted conditions (Bækgaard Nielsen et al 2017).e.g., $\uparrow P_{Cl}$ would not appreciably delay the onset of myogenic firing in ischemic (DMD) fibers. By contrast, in such fibers, $\downarrow P_{Na}$ would hyperpolarize V_m , thereby lowering endplate excitability, and would also delay myogenic firing.

Deeply ischemic LA-CD has the same P_{Na} as SM-CD but its higher $[Na^+]_i$ and slightly depolarized V_{rest} (**Table 2**) slightly reduce its Na^+ driving force. As for SM-CD, the anoxic rundown pace of LA-CD is P_{Na} -limited. As seen in **Figure 2Ci**, ectopic firing commences in LA-CD at 22.0 minutes (22.7 for SM-CD). During ectopic firing LA-CD’s AP-associated currents (**Figure 2Cii**) differ inconsequentially from those (not shown) of SM-CD. Nevertheless, LA-CD can recover provided its pumps (30%) are restored before ectopic firing ends (**Figure 2Ciii**); as expected, recovery is slower than for SM-CD (which gets 0% $\rightarrow$ 100%, not 0% $\rightarrow$ 30%). Consistent with their syncytial SMF geometry, chronically-ischemic DMD fibers recovering after a comparable bout of transient functional ischemia/anoxia would exhibit considerably more prolonged recovery periods than LA-CD.

“Too little Na^+ -pumping” alone could explain chronic Na^+ -overload in DMD

While LA-CD’s elevated $[Na^+]_i$ already hints that ischemia alone (“too little Na^+ -pumping”) might suffice to explain *mdx*-level Na^+ -overloads, pump-strength effects need to be examined systematically.

Excitable P-L/D systems have non-linear driving forces and two interacting non-linear Na^+ fluxes ($I_{pump}([Na^+]_i)$ and $I_{Nav}(V_m)$). Even for ultra-simple SM-CD, therefore, predicting/describing how ion homeostasis plays out across pump-strengths requires graphical analysis. Joos & Morris (submitted) showed that across its full pump-strength range (up-regulated several-fold down to anoxic), SM-CD, like

other excitable P-L/D systems (e.g., CN-CD; Dijkstra et al 2016; Hübel et al 2014), exhibits bistability, i.e., discontinuous physiological and pathological steady-state continua accessed via two unstable thresholds (see **Figure 3A**).

For chronically ischemic DMD however, only the ischemic-but-viable physiological steady-states (**Figure 3B,C**) are relevant. For V_m (pump-strength) (**Figure 3A**) only, the full bifurcation plot is provided to give context for partial plots **Figure 3B,C**. Beyond the saddle-node(**X**), ectopic firing induced Ca^{2+} -necrosis would render the pathological steady-state continuum (**Figure 3A**) irrelevant for DMD (in *mdx*, ectopic Ca^{2+} -mediated contractures destroy the fibers (Claflin and Brooks, 2008)).

For DMD patients, $[Na^+]_i$ is a measurable ion homeostatic parameter, so **Figure 3B** emphasizes the physiological steady-state continuum for $[Na^+]_i$ (pump-strength) in the ischemic (<100%) range. On these plots, our DMD mimic, LA-CD, essentially corresponds to 30%. Thanks to its [small I_{NaLeak}], ischemic SM-CD maintains pump-reserve>1-fold until pump-strength drops to ~8%, below which (i.e. at its saddle-node(**X**)), the system destabilizes. If (as per **Figure 2A→B**) P_{Na} were to decrease, would hold off to an even lower pump-strength. SM-CD could be considered “chronically Na^+ -overloaded” from ~20-15% down to 8%. Thus, P-L/D analysis indicates that, in principle, DMD/*mdx* chronic Na^+ -overload could arise solely from “too-little- Na^+ -pumping.”

When severe ischemia subsides, recovery by Donnan dominated SM-CD is an incomparably more robust than for Pump-Leak dominated neurons (Joos & Morris, submitted) where the saddle-node is at 65% pump-strength; if pump-strength falls to, say, 64%, only a pump-strength increase $\geq 181\%$ allows for recovery (Dijkstra et al 2016). For SM-CD, however, any $\uparrow$ blood-flow (i.e., any $\uparrow$ pump-strength to a level >8%) contributes to recovery.

For DMD ischemia, therapeutic avenues for $\uparrow$ pump-strength include counteracting vascular insufficiency (e.g., angiogenesis (Podkalicka et al 2019)), $\uparrow$ vasodilation (e.g., NO-reagents (Patel et al 2018)) and $\uparrow$ functional pump-density (Breitenbach et al 2016; Glesmer et al 2017; Raman et al 2017).

The danger zone for DMD

For physiological SM-CD steady-states in the ischemic range, **Figure 3C** assembles the P-L/D parameter plots. This shows that above ~40% pump-strength, SM-CD is extremely robust, while below ~20%, system deterioration is steep. In this “danger zone” V_m depolarizes appreciably, while $[Na^+]_i$ and $[Cl^-]_i$ rise and $[K^+]_i$ falls precipitously (note absolute amounts). Because $P_K \gg P_{Na}$, concurrent Na^+ -loading/ K^+ -depletion largely preserve cytoplasmic electroneutrality. Even at the saddle-node, where SM-CD $[Na^+]_i=88$ mM, Cl^- -loading is so minimal that swelling is only ~4.5% ($2000\mu m^3 \rightarrow \sim 2090\mu m^3$). As Vol_{cell} increases, impermeant anions dilute slightly ($149.6mM \rightarrow 143.1mM$ for 100% $\rightarrow$ saddle-node; not shown). Thus, in spite of [big P_{Cl}], chronic ischemia-induced Na^+ -overloads are almost “non-osmotic”. This accords with recent MRI findings from DMD patients’ whose muscles show chronic Na^+ -overload even in the absence of “water T_2 alterations” (Gerhalter et al 2019). Any demonstrably-swollen Na^+ -overloaded fibers in patients would likely be experiencing myogenic APs.

Though computational ATP is never limiting in our analyses, note the situation below 20% pump-strength; as $[Na^+]_i$ (pump-strength) rises steeply, ATP-consumption declines abruptly. As detailed further in the **Figure 3** legend, this can equivalently be described as a steep decline in hyperpolarizing I_{pump} ($= -I_{NaLeak}$) in this zone; briefly, falling ATP-consumption reflects pump saturation, E_{Na} dissipation and specifics of the P-L/D system’s $I_{pump}/[Na^+]_i$ feed-back interactions.

P-L/D bifurcation analysis of SM-CD thus shows that deep ischemia (“too little Na^+ -pumping”) yields chronic “non-osmotic” Na^+ -overload at levels that are tolerated because the Donnan dominated system’s “control valve” (its extremely small P_{Na}) has facilitated its large pump-reserve.

This in no way implies that damage-induced Na^+ -leaks do not also contribute to DMD Na^+ -overload, only that ischemia, if sufficiently deep, would suffice. For “milder” ischemia (say, pump-

strengths>25%) chronic Na⁺-overload would (by elimination) also reflect Na⁺-leaks and/or over-active Na⁺-transporters. Bleb-damage sections below address leaky Na⁺-permeant channels.

The concentration of extracellular Donnan effector is invariant here. However, as Mehta et al (2008) indicate, for very small pump-reserve, this parameter would affect steady-state. In the danger zone, pump-reserve is increasingly marginal. Since Coles et al (2020) report protease-release of extracellular matrix proteins in exercised *mdx* fibers, extracellular Donnan effector impacts (influenced, probably, by ischemia-related ΔpH (Hagsberg 1983)) could be worth revisiting.

Sleep, diaphragm fibers and the danger zone

Though DMD patients avoid over-exertion, even their sleep holds dangers (Hartman et al 2020). Skeletal muscle tissue is no known to be directly involved (Ehlen et al 2017) in generating sleep rhythms, including variations in SMF glucose homeostasis and oxidative capacity (Harfmann et al 2016; van Moorsel et al 2016). During REM sleep, DMD patients' impaired sleep and respiratory profiles become acutely compromised (Nozoe et al 2015). For already-ischemic DMD diaphragm fibers, diminished energy status during sleep could take them treacherously far into the danger zone. For healthy steady-states, pump-model specifics matter little (Düsterwald et al 2018). However, for deeply ischemic excitable cells, pump-specifics that affect a P-L/D danger zone's inflection region and steepness could be important. Future SM-CD refinements focusing on the DMD danger zone might include using SMF-specific pump model, perhaps incorporating dystrophic fibers' pump characteristics (Hakimjavadi et al 2018; Kravtsova et al 2020). Similarly, since V_{rest} is chronically depolarized in the danger zone, Nav gating particulars (e.g., Filatov et al 2005) could be added to accurately depict fiber excitability.

DMD bleb-damage: "too much Na⁺-leak"

In SMFs, $P_K \gg P_{Na}$. Therefore, the major consequence of any "leaky cation channel" will be augmented P_{Na} . Specifically, in SM-CD and LA-CD, $P_K:P_{Na}$ is 1.00:0.03 so if an SMF cation channel with, say, $P_K:P_{Na}=1:1$ over-activated enough to double P_{Na} (100% increase), P_K would increase only 3%. This is relevant because in many excitable cells, a hormonally-regulated cation channel, NALCN (**NA Leak Channel Non-selective**) contributes to resting P_{Na} (Lu et al 2007; Cochet-Bissuel et al 2104; Lutas et al 2016; Philippart and Khaliq, 2018; Reini et al 2018). No NALCN is detected in SMFs, but other cation channels (Metzger et al 2020) might contribute to P_{Na} and if over-active, they would be problematic. In *mdx* fibers, over-active mechanosensitive cation channels (unidentified) and AChRs are posited routes for pathological Ca²⁺-entry (Franco and Lansman 1990; Carlson and Officer 1996; Yeung et al 2005; Lansman 2015; Ward et al 2018). If so, unavoidably, they are also "Na⁺-leaks". The mechanosensitive channel is a suggested DMD therapeutic target for GsMtx4-related peptides (Ward et al 2018; Gnanasambandam et al 2017).

The unspecified computational "leaky cation channel" modeled here could represent either, both, or neither of these cation channels but it is assigned $P_K:P_{Na}=1.11:1$, the ratio of SMF AChRs (Hille 2001). AChRs are abundant, identified, and implicated as leaky in *mdx* (Carlson and Officer 1996). They are also mechanosensitive, GsMtx4-inhibited (Pan et al 2012). Plausibly, their spontaneous activation (Jackson et al 1990) might increase in damaged junctional *mdx* fiber sarcolemma (Pratt et al 2015).

Healthy or dystrophic, SMFs' biggest Na⁺ influxes are via Nav1.4 channels (Fu et al 2011). Nav1.4 binds syntrophin and thence dystrophin (whether utrophin can substitute is unknown (Gee et al 1998; Albesa et al 2011)). Sarcolemmal Nav1.4 density is somewhat low in *mdx* (Ribaux et al 2001; Hirn et al 2008) but Nav1.4 gating appears normal (Mathes et al 1991). Hirn et al (2008) showed that for 3 days after mechanically-traumatic fiber isolation, 3 nM tetrodotoxin protects *mdx* fibers from Na⁺-loading and die-off (**Figure 1B**). In DMD sarcolemma, Na⁺-leak through Nav1.4 channels would likely take the form of left-shifted window-current from Nav channels in bleb-damaged sarcolemma (see "Nav-CLS", **Figure 1D**;

Morris et al 2012a,b). The clinical Nav-antagonist, ranolazine, is a proposed DMD therapeutic (Burr et al 2014; Lorusso et al 2019).

The next sections show SM-CD (and other models) dealing with both classes of Na⁺-leak (Nav-CLS, over-active cation channels).

Bleb-damage: Nav-CLS is treacherous but cannot explain chronic Na⁺-overload

Figure 4 shows P-L/D responses to increasingly intense Nav-CLS (**Figure 1D** and **Methods**) from bleb-damage to a fraction (0.3) of the Nav-bearing sarcolemma. As detailed in the figure legend, CN-CD (**Figure 4A**) and SM-CD (**Figure 4B**) are compared. Since Pump-strength=100%, P-L/D feedback is initially able to counteract SM-CD's damage-induced ectopic firing. This differs from ectopic firing due to anoxia (**Figure 2A**) or to extreme-ischemia (Joos & Morris submitted, their Fig 7Aii) which inexorably ends in cytotoxic swelling. Until the system destabilizes at Nav-CLS(0.3)~30 mV, this Nav-CLS-induced ectopic firing causes minimal swelling. The resultant ectopic contractures (e.g. at Nav-CLS(0.3)=28 mV; **Figure 4Bii**) would, however, be very problematic *in situ*.

Other DMD patho-phenomena here include subthreshold hypersensitivity (**4Bi** top panel) that would foster myogenic APs. This likely contributes to DMD's erratic spontaneous contractility (Ishpekova et al 1999; Nojszewska et al 2017). Single fibre EMG recordings from advanced-stage DMD patients show bizarre repetitive discharge bursts (Trontelj and Stålberg, 1983; see Yu et al 2012). In *mdx* muscles, electromyography of abnormal spontaneous potentials and complex repetitive discharges resemble the muscle discharges of boys with DMD (Carter et al 1992; Han et al 2006). Where there is Nav-CLS-induced hypersensitivity, erratic myogenic APs bouts are expected if the damaged dystrophic sarcolemma also has leaky" cation-channels that activate intermittently, depolarizing V_m (Lansman 2015; and see next section).

The exquisitely narrow Nav-CLS damage range between zero Na⁺-loading and catastrophic instability ([Na⁺]_i panel, **Figure 4Bi**) makes Nav-leak an implausible stand-alone explanation for DMD's chronic Na⁺-overload. Nevertheless, the considerable danger posed by subthreshold Nav-CLS effects (hypersensitivity) and myogenic firing make it important to determine if slow-inactivation of Nav1.4 (Webb et al 2009; Morris et al 2012A,B; Lukacs et al 2018) protects SMFs during the membrane repair/regeneration processes critical for DMD longevity (Baumann et al 2020). However, even for otherwise healthy fibers recovering from overexertion-tears (Dahlmann et al 2016; Hotfiel et al 2018; Hody et al 2019) this seems not to have been examined.

Bleb-damage: depolarizing and Na⁺-loading effects of leaky cation channels

In **Figure 5** cation-channels are activated (P_{Na}-component increased by 0.125 μm³/s; see legend and **Methods**) for 4 minutes in SM-CD, LA-CD, and in WD-CD (compare in **Table 2**).

At 30% pump-strength, LA-CD offers insight for DMD. In both LA-CD and in SM-CD the depolarizations would be readily measurable *in vitro*. But while SM-CD's attendant ↑[Na⁺]_i (~0.5 mM) would not measurably alter "Na⁺-overload", the rise in LA-CD (to [Na⁺]_i>8 mM) puts it the range of *mdx* Na⁺-overload (see **Table 1**; item **8**). Note that ~2/3 of the LA-CD Na⁺-overload is attributable, however, to "too little Na⁺-pumping" with ~1/3 from "too much Na⁺-leak". Increasing cation-leak in SM-CD to 0.250 μm³/s (SM-CD-P_{Na} ↑83%) is strongly depolarizing (-86 mV→-77.5 mV), but [Na⁺]_i still rises only 3.7→4.5 mM (not shown).

Such calculations indicate that otherwise-healthy fibers (SM-CD) harboring leaky cation channels that doubled P_{Na}, would depolarize by ~10 mV, with little ↑[Na⁺]_i (<2 mM) or swelling (<1%) whereas DMD-like fibers (LA-CD), though minimally swollen, would become measurably Na⁺-overloaded, depolarized and vulnerable to myogenic discharge.

Another menace from “leaky cation channels” relates to anoxic bouts: the $\uparrow P_{Na}$ accelerates rundown then compromises recovery ($\uparrow I_{NaLeak} \rightarrow \downarrow \text{pump-reserve}$). In *mdx* fibers stretch-injury can cause prolonged (non-lethal) depolarization (Baumann et al 2020) but the identity of Na^+ -permeant paths rendered leaky by injurious stretch are unknown.

Chronic ischemia + repetitive stimulation + bleb-damage Na^+ -leaks

Healthy rat soleus fibers can fire 1200 APs (120 Hz, 10 seconds) then briskly return to steady-state (see Clausen, 2015); 100% pump-strength SM-CD recapitulates this stress-test (Joos & Morris submitted) and **Figure 6A** shows that even deeply ischemic LA-CD handle this challenge well, though its recovery is slower by several minutes. The robustness of LA-CD’s response suggests that, *in situ*, comparably ischemic SMFs would have adequate pump-reserve to support Na^+ -transporters since end-plate triggered APs would occur at much lower frequencies than the stress-test.

But dystrophic ischemic fibers might also have bleb-damage Na^+ -leaks. Inflicted with, say, 10 mV Nav-CLS(0.3), or with sufficient cation channel leak to double its P_{Na} , LA-CD still passes the stress-test and slowly recovers to its leaky ischemic steady-state (not shown). Robustness, however, has limits as seen in **Figure 6Bi**. There, ischemic LA-CD is inflicted with both the above-mentioned Na^+ -leaks then stress-tested. Due to $\uparrow P_{Na}$, the steady-state (the quiescent state prior to “t=0”) is depolarized by ~12 mV, but the ischemic doubly-traumatized system successfully fires 1200 APs. However, once EPSCs stop at 10 s, (**Figure 6Bii**) it becomes evident that some threshold has been crossed. The system is not on a recovery trajectory. It fires ectopic APs (~20 Hz) of diminishing amplitude; they cease abruptly at ~60 seconds. Overstimulated, ischemic, bleb-damaged LA-CD (still consuming ATP) converges toward a pathological steady-state. P-L/D parameter trajectories (not shown, but as per **Figure 2Ci**) reveal a cytotoxically-swelling system. An excitable-P-L/D bistability scenario has occurred. At some point, while firing 1200 APs, the impaired system destabilized, meaning that it encountered, in parameter space, an unstable threshold beyond which the hyperpolarizing I_{pump} (associated with 30% maximum-strength) was insufficient to counteract total depolarizing $I_{NaLeaks}$. *In situ* contracture-induced Ca^{2+} -necrosis would likely occur before cytotoxic swelling developed. Slow inactivation of Nav1.4 could help forestall destabilization.

Na^+ -overload and DMD multiple-jeopardy

Ischemia and bleb-damage typically co-exist for DMD fibers. With ^{23}Na -MRI monitoring of patients in mind, we survey (**Figure 7**) their predicted simultaneous impacts on chronic Na^+ -overload. **Figure 7A** (left Y-axis, magenta) plots the smallest pump-strength that ensures SM-CD viability (conservatively defined as “no myogenic firing”) with increasing bleb-damage intensity (X-axis; Nav-CLS(0.3)). The right Y-axis and the inset show the corresponding steady-state $[Na^+]_i$ and Vol_{cell} .

Figure 7B replots **7A** minus the magenta “pump-strength” curve ($[Na^+]_i$ at 10%, 15%, 30% from **7A** are flagged). The “safe”/“lethal” $[Na^+]_i$ partition-line emphasizes, qualitatively, how bleb-damage reduces a fiber’s tolerance of $[Na^+]_i$ -overload. For fibers in DMD-patient muscles, co-ordinates would scatter within the safe zone. Toward the left, a safe fiber near the Na^+ -line would be particularly vulnerable to, say, Ca^{2+} -necrosis via reverse Na^+/Ca^{2+} -exchange as per Burr et al (2014). Toward the right, safe fibers would risk excitation-triggered release of Ca^{2+} -stores (e.g., Claflin and Brooks, 2008). **Figure 7C** is a “sketch” (no specific computations) depicting how leaky-cation channels further imperil survivability.

Human myoplasmic $[Na^+]_i$ (based on ^{23}Na -MRI) is evidently higher than in mice (**Table 1**). Hammon et al (2015) emphasize how ^{23}Na -MRI could “be used to gain new insights into {human} Na^+ homeostasis, presumably leading to better comprehension of pathophysiology”. An anecdotal proof-of-principle study (Dahlmann et al 2016) encourages that view: in a young athlete, muscle injury and recovery (non-injured contralateral=control) was monitored via ^{23}Na -MRI. The outcome (in mM) for $[Na^+]_i$.

control/ $[\text{Na}^+]_{\text{i-injured}}$: time=0 18/44; 2 weeks 19/38; 2 months 19/22. Congruent with SM-CD and LA-CD anoxic rundown/recovery (**Figure 2B,Cii,ii,E**), Hammon et al (2015) showed that during anaerobic exercise, muscle exhibits a water-independent (i.e. non-osmotic) $\uparrow[\text{Na}^+]_{\text{i}}$ and, during recovery, a $\downarrow[\text{Na}^+]_{\text{i}}$. Advances in cell physiological-level instrumentation, too, are will deepen P-L/D investigations of SMFs: a four-electrode method (Heiny et al 2019) now makes it possible to follow multi-ion dynamics and transport processes under voltage- or current-clamp, even monitoring $[\text{Cl}^-]_{\text{i}}$ accurately (DiFranco et al 2019). With a flood of observations relevant to DMD fiber ion homeostasis expected, the P-L/D theoretical framework presented here should help make those findings, plus results from the plethora of DMD molecular therapies trails now underway (Verhaart and Aartsma-Rus 2019), more comprehensible, while also suggesting additional lines of inquiry.

DISCUSSION

Progressive fiber-loss renders DMD patients non-ambulatory by their early teens. 10-15 years later, weakened diaphragm and thoracic muscles cause respiratory failure and premature death (Allen et al 2016; Gerhalter et al 2019). Given the multitude of primary and secondary defects besetting DMD SMFs (e.g., Murphy et al 2019), fiber survival for weeks let alone decades is remarkable and begs explanation. We therefore mostly ask not “what is wrong with DMD fibers?” but “what is right with DMD fibers?”

An extant model for excitable SMF ion homeostasis (Fraser et al 2011), though powerful and rigorous, is not readily implemented and has seen little uptake for either healthy or ailing fibers. However, guided by the Fraser and Huang (2004) charge difference approach (and SMF permeability ratios) we modified the more recent ion homeostasis model, CN-CD (devised to probe cortical spreading depression (Dijkstra et al 2016)) to obtain SM-CD, our minimal model for ion homeostasis in excitable SMFs (Joos and Morris, submitted). Though both models exhibit patho-physiologically critical (lethal) threshold phenomena (i.e., pump-strength points below which homeostatic insufficiency elicits lethal spontaneous firing), the distinctive Donnan dominated P-L/D strategy of SMFs, safeguards SMFs through short term emergencies and the chronic state-of-emergency that is DMD.

Overview: SMFs’ Donnan dominated P-L/D process

SMFs’ energy-efficient hyperpolarized steady-state is achieved by a P-L/D process that is Donnan dominated ($[\text{big } P_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$). To address intermittent overexertion/trauma/misadventure, SMFs maintain an ample pump-reserve. To keep ever-present Donnan swelling forces at bay, SMFs keep P_{Na} extremely small. The resulting $[\text{small } I_{\text{Naleak}}]$ minimizes $3\text{Na}^+/2\text{K}^+$ -pump expenditures, fosters the ample pump-reserve, keeps anoxic rundown slow and enables the hyperpolarized V_{rest} . Given $[\text{small } I_{\text{Naleak}}]$, SMFs can, for no-added-cost, stabilize V_{rest} at or near E_{Cl} by exploiting the low-impedance “battery” available through the $[\text{big } P_{\text{Cl}}]/\text{Donnan-effector}$ pairing.

SMF ion homeostasis ($[\text{big } P_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$) confronts DMD as follows:

1. Chronic ischemia is tolerated because $[\text{small } I_{\text{Naleak}}] \rightarrow \text{low steady-state ATP-consumption}$.
2. As ischemic low duty-cycle excitable cells (i.e., mostly at V_{rest}), DMD fibers benefit from the no-cost low-impedance $[\text{big } P_{\text{Cl}}]/\text{Donnan-effector}$ “battery” that stabilizes their (cheaply) hyperpolarized V_{rest} .
3. Bouts of functional ischemia and/or other transient episodes of anoxia/ischemia are survivable because $[\text{small } I_{\text{Naleak}}] \rightarrow (\text{slow rundown})$ with minimal swelling. How? During rundown, since $P_{\text{K}} \gg P_{\text{Na}}$, and V_{m} closely tracks E_{Cl} , electroneutrality and osmo-balance are maintained while $[\text{Na}^+ + \text{Cl}^- + \text{H}_2\text{O}]_{\text{entry}}$ stays inconsequential.
4. The chronic Na^+ -overload of DMD is “non-osmotic” in nature (same explanation as in 3).
5. The SMF pump-reserve is so big that even in deeply ischemic (DMD-like 30% pump-strength) SM-CD it exceeds that of 100% “CN-CD neurons” it can support fiber recovery (albeit, more slowly, and within

limits) after overstimulation and transient bouts of anoxia and when sarcolemmal damage induces Na^+ -leaks.

6. The large pump-reserve can redress transient the Na -loading associated with the intermittent myogenic firing (triggered, e.g., by sporadic Na^+ -leaks in DMD-damaged sarcolemma).

7. Though pump-reserve “runs out” only at an exceeding low pump-strength ($\sim 8\%$ as modeled here), diaphragm fibers (they must regularly fire APs) would destabilize at a somewhat higher pump-strength.

8. “Leaky” Na^+ -permeant channels and E_{Na} -depleting Na^+ -transporters (the latter not modeled here) would exacerbate chronic Na^+ -overload but would not be its sole cause.

9. “Leaky” Nav and cation channels are expected in the bleb-damaged sarcolemma of DMD fibers. Modeling them in the ischemic P-L/D setting shows how they could influence various DMD-pathophenomena (chronic and/or sporadic depolarization, Na^+ -overload, erratic myogenic firing).

10. Though the Ca^{2+} -necrosis conditions of DMD fibers are difficult to pinpoint, they emerge in our SM-CD based analysis as P-L/D thresholds. They are encountered when severe ischemia and/or severe Na^+ -loading (Na^+ -leaks, excess APs) push the P-L/D system to an unstable point in parameter space (i.e., where system-values make it impossible for hyperpolarizing I_{pump} to re-establish a stable physiological state). The emphasis here on “severe” bespeaks the robustness of even deeply compromised DMD fibers. Diverse “only-just” lethally-destabilizing circumstances (see {} brackets) were simulated here, including:

- i. (extreme ischemia): 7% pump-strength {Safe at 9%}.
- ii. (extreme bleb-damage): 100% pump-strength but Nav-CLS(0.3)=30 mV {Safe at 24 mV}.
- iii. (deep ischemia + over-stimulation + ischemia + membrane damage):
30% pump-strength + APs @120 Hz for 10 s + Nav-CLS(0.3)=30 mV + $2XP_{\text{Na}}$
{Safe with $1XP_{\text{Na}}$ OR with Nav-CLS(0.3)=0 mV}.

An aside

Since a P_{Cl} (not P_{K}) dominated resting conductance is mandatory for mammalian SMF resilience, and since some aspects of exertion-related DMD pathology are well-modeled, biologically, via *C elegans* body wall musculature (Hughes et al 2019), we note that resting conductance in nematode muscle is P_{Cl} -dominated (Brading & Caldwell, 1971) and that nematodes have ClC channels (Jentsh and Pusch, 2018).

DMD fibers’ chronic state of emergency

Vertebrate SMFs evolved as long-lived syncytial fibers reliant on the superlative emergency preparedness afforded by a cheaply-maintained pump-reserve far in excess of requirements for normal operation. SMFs thus survive the intermittent ion homeostatic emergencies to which working muscles are prey. These emergencies could involve temporary (minutes to hours) blood-flow restrictions, starvation, over-exertion, tissue trauma, sarcolemmal-damage, sarcolemmal-tearing. Tourniquets are tolerated for prolonged periods (Zhang et al 2020) but toleration of ischemia has a limit. Compartment syndrome (a massive, generally sudden, situation of pressure-impeded blood flow, often compounded by tissue damage) represents an emergency circumstance too extreme even for SMFs, barring surgical intervention that restores blood flow before an irreversible (poorly characterized) threshold is reached (Johnstone and Ball 2019).

DMD fibers, in effect, exist in a state of “chronic emergency”; this is debilitating – exertion is deleterious for DMD patients - yet, thanks to [big P_{Cl}][small I_{NaLeak}] ion homeostasis, non-lethal.

For DMD fibers, I_{pumpmax} is chronically too small and sarcolemmal damage can make operational I_{NaLeak} too large. Modeling here repeatedly highlights the need to identify skeletal muscle’s overlooked P_{Na} , then to learn if/how it can be safely reduced to counteract these pathological deficits.

The activity regimes of, say, diaphragm versus limb fast-twitch fibers differ markedly. SM-CD is generic. Its clinical utility could be amplified, as appropriate, by modifying pump, leak and excitability characteristics for particular fiber-subtypes. As more molecular, genetic and pharmacologic therapies

for DMD come to the clinic (Verart and Aartsma-Rus 2019; Meng et al 2020), non-invasive MRI-monitoring of myoplasmic $[Na^+]$ and other ion homeostatic indicators (Gerthaler et al 2019) will increasingly be used to gauge treatment efficacy. Even in its present utterly basic form, the SM-CD theoretical framework provides fresh insights into the cell physiological meaning of clinical indicators, and helps explain DMD fiber resilience and vulnerability. P-L/D modeling of SMFs facilitates clinical interpretation and generates questions, the most pressing raised here being:

1. What proteins underlie SMFs' critical small P_{Na} ?
2. Could DMD fiber longevity be extended by diminishing P_{Na} ?

METHODS

A Pump-Leak/Donnan (P-L/D) model for SMFs

Skeletal muscle fiber (SMF) ion homeostasis is modeled (SM-CD) by a single compartment limited by a semipermeable membrane in an "infinite" (fixed concentrations) extracellular volume (**Table 2**). SM-CD constitutes a single P-L/D "ion homeostatic unit"; an actual multinucleate SMF would be comprised of hundreds or even thousands of such units (depending on sarcolemma area) and, as per Joos and Morris (submitted) would likely have a smaller SA/V (surface area to volume) ratio than SM-CD (thus, slower $\Delta[ion]_i$ dynamics). The SM-CD membrane encloses a fixed quantity of Donnan effectors: A^- , impermeant monovalent anions. The extracellular medium has a fixed A^- concentration. The membrane is permeable to Na^+ , K^+ , and Cl^- ions whose extracellular concentrations are fixed. SM-CD has the same physical characteristics as the Dijkstra et al (2016) neuronal cell, CN-CD (see **Table 2**): a resting cell volume (Vol_{cell}) of $2000 \mu m^3$ and a constant C_m corresponding to a SA of $2000 \mu m^2$. As such, resting state cells are flaccid (i.e., non-spherical). Lipid bilayers tolerate little lateral expansion, but unless a model-cell inflated to spherical its membrane would not be subject to tension and rupture (see **Table 2**). The permeation pathways of SM-CD and CN-CD (given below) include "resting leak conductances" (i.e., permeation pathways) for $I_{Na^+}^L$, $I_{K^+}^L$, and $I_{Cl^-}^L$ and voltage-gated conductances (permeation pathways) for a transient sodium current, $I_{Na^+}^T$, for delayed rectifier potassium current, $I_{K^+}^D$, and (CN-CD only) a voltage-dependent chloride current, $I_{Cl^-}^G$. Driving forces acting on ions are, in all cases, electrodiffusive, as depicted by Goldman Hodgkin Katz (GHK) current equations (Hille, 2001). The same $3Na^+_{out}/2K^+_{in}$ ATPase pump model (Hamada et al 2003) is used throughout. It produces hyperpolarizing current

$$I_{pump}^{Na^+} = 3I_{pump} = -\frac{3}{2}I_{pump}^{K^+}, \quad (1)$$

in response to the intracellular $[Na^+]$ (as per **Figure 4**). In CN-CD only there is a K^+/Cl^- co-transporter for ion flow, J_{KCl} .

Since animal cells cannot sustain osmotic pressures, intra/extracellular osmolyte concentration inequalities produce a H_2O flow till osmotic balance is restored; this results in Vol_{cell} changes at rates set by the smaller of the two components giving rise, at any time, to osmotic loading, i.e. to net $[Na^+ + Cl^-]$ entry.

Choice of leak permeabilities

Whereas CN-CD is precisely the Dijkstra et al (2016) model, the SMF version (SM-CD) has a leak permeability ratio $P_{Na^+}^L : P_{K^+}^L : P_{Cl^-}^L$ broadly consistent with Fraser and Huang (2004); for more detail see

Setting V_{rest} in SM-CD and other CD models below.

GHK driving forces

Currents through open channels (permeability pathways), ion-specific or not, are modeled with the GHK formulation (Hille, 2001). For ion $X = Na^+$, K^+ , or Cl^- , the GHK current is given by:

$$I_{GHK}(X, P_X, V) = P_X(z_X F) \frac{z_X FV}{RT} \frac{[X]_i - [X]_e \exp\left(-\frac{z_X FV}{RT}\right)}{1 - \exp\left(-\frac{z_X FV}{RT}\right)}, \quad (2)$$

where P_X is the permeability, z_X the valence, and $[X]_i$ and $[X]_e$ the intra- and extra-cellular concentrations of X respectively.

Leak currents

“Leak” permeability mechanisms use the GHK formulation:

$$I_X^L = I_{GHK}(X, P_X^L, V), \quad (3)$$

where X is either Na^+ , K^+ or Cl^- . Leak permeability (P_X^L) values were adjusted for model variants used here (**Table 2**) in the context of appropriate setting of V_{rest} .

Cation channel currents

For non-selective cation channels, we use a $P_K:P_{Na}$ ratio of 1:1.11 and the formulation:

$$I_{CatLeak}(t) = I_{GHK}(Na^+, P_{Na^+}^{CatLeak}, V) + I_{GHK}(K^+, P_{K^+}^{CatLeak}, V). \quad (4)$$

In the present models, cation channel leaks do not contribute to healthy steady-states – they are either transient stimulatory currents through SMF-endplate type AChR channels, or pathological leaks (hence “leaky” cation channels).

Transient voltage-gated Na^+ current

$$I_{Na^+}^T = m^3 h I_{GHK}(Na^+, P_{Na^+}^T, V). \quad (5)$$

$P_{Na^+}^T$ is the maximal membrane permeability to Na^+ through a V-gated channel (operating in a Hodgkin-Huxley (H-H) fashion). m is the H-H Na^+ channel activation/deactivation gating variable and h is the H-H Na^+ channel inactivation/recovery gating variable. The current’s driving force also follows the GHK form of **Equation 1**.

Delayed rectifier K^+ current

$$I_{K^+}^D = n^2 I_{GHK}(K^+, P_{K^+}^D, V). \quad (6)$$

$P_{K^+}^D$ is the maximal membrane permeability of K^+ through a V-gated channel (operating in a H-H fashion); n is the delayed rectifier K^+ channel activation/deactivation gate variable.

Voltage-dependent gating

The non-dimensional gating parameters m , h , n evolve in time according to:

$$\frac{dq}{dt} = \alpha_q(V)(1 - q) - \beta_q(V)q, \quad (7)$$

where $q \in \{m, h, n\}$ are as defined above, and the voltage dependent $\alpha_q(V)$ and $\beta_q(V)$ refer, for m and n to gate activation and deactivation and, for h to inactivation and recovery from inactivation. **Table 3** gives the voltage dependences for the relevant rate constants.

Voltage-dependent Cl⁻ current

The SLC26A11 ion exchanger based voltage-dependent Cl⁻ conductance (Rungta et al, 2015) is as described by Dijkstra et al (2016):

$$I_{Cl^-}^{SLC} = \frac{I_{GHK}(Cl^-, P_{Cl^-}^{SLC}, V)}{1 + \exp\left(-\frac{V + 10 \text{ mV}}{10 \text{ mV}}\right)} \quad (8)$$

Except in CN-CD and MN-CD, this pathological cortical neuron specific conductance is set at zero.

K⁺/Cl⁻ cotransporter

This cotransporter (strength U_{KCl}) is present only in CN-CD (it is not evident in skeletal muscle; Pedersen et al 2016) where (as per Dijkstra et al 2016) it is given by

$$J_{KCl} = U_{KCl} \frac{RT}{F} \ln \left(\frac{[K^+]_i [Cl^-]_i}{[K^+]_e [Cl^-]_e} \right) \quad (9)$$

(except that there is an error in the log term of Eq. (6) in Dijkstra et al 2016).

3Na⁺/2K⁺ ATPase pump current

The electrogenic 3Na⁺(out)/2K⁺(in) ATPase modeled here is that of Hamada et al (2003):

$$\frac{I_{pump}}{Q_{pump}} = \frac{0.62}{1 + \left(\frac{6.7 \text{ mM}}{[Na^+]_i}\right)^3} + \frac{0.38}{1 + \left(\frac{67.6 \text{ mM}}{[Na^+]_i}\right)^3} \quad (10)$$

where

$$I_{pump} = I_{pump}^{Na^+} + I_{pump}^{K^+} \text{ or } I_{pump} = \frac{1}{3} I_{pump}^{Na^+} = -\frac{1}{2} I_{pump}^{K^+} \quad (11)$$

Thus I_{pump} signifies a net hyperpolarizing Na⁺ outflow. The Hamada et al (2003) formulation is appropriate for models that assume an invariant extracellular medium. It depicts the pump's two intracellular Na⁺-binding sites (with ~10-fold different affinities) but has no term for the extracellular K⁺-binding site. Here, pump-strength (Q_{pump}) is varied in many computations (e.g., it is set to zero to depict anoxia, diminished towards zero to depict ischemia and multiplied for up-regulation).

ATP-Consumption

ATP-consumption by the Na⁺/K⁺ ATPase pump in the all the CD models is given as

$$ATP / s = \frac{I_{pump}}{eN_A} \quad (12)$$

where e is the electronic charge (1.6×10^{-19} Coul) and N_A is the Avogadro number. In other words, I_{pump} of 1 pA is equivalent to an ATP-consumption of 10.38 amol/s.

Cell Volume

Cellular swelling is driven by an influx of water at rates that depend on the transmembrane osmotic gradient. The rate of change of cell volume, Vol_{cell} , due to water influx is given by:

$$\frac{dVol_{cell}}{dt} = J_{H_2O} = L_{H_2O} \Delta \pi \quad (13)$$

where $\Delta\pi = RT([S]_i - [S]_e)$, and $[S]_i$ and $[S]_e$ denote total concentrations of intra- and extracellular solutes and L_{H_2O} is the effective membrane water permeability. This equilibration is typically assumed to be nearly instantaneous relative to the ion flows (Fraser and Huang, 2004; Dijkstra et al 2016; Kay, 2017; though see also Dmitriev et al 2019). Accordingly, in modeling here, osmotic ion fluxes, not the H_2O fluxes what limit the rate of osmotic swelling or shrinkage.

Nernst potentials

The equilibrium potential (Nernst potential) for each ion

$$E_x = -\frac{z_x RT}{F} \log\left(\frac{[X]_i}{[X]_e}\right), \quad (14)$$

where X is either Na^+ , K^+ , or Cl^- ions and z_X is the valence of each ion.

Charge difference and membrane potential

Because CD models keep track of the absolute number of ions flowing across the cell membrane (of capacitance C_m) no differential equation is needed for V_m . Instead, an accounting equation is used, made simple because the extracellular space is kept neutral:

$$V_m = \frac{F}{C_m} \left(N_i^{Na^+} + N_i^{K^+} - N_i^{Cl^-} - N_i^{A^-} \right) = \frac{F}{C_m} \left(dN_{Na^+} + dN_{K^+} - dN_{Cl^-} - dN_{A^-} \right), \quad (15)$$

where $dN_x = N_i^x - N_{i,0}^x$ is the difference in the number of ions of species x , between its present value N_i^x and a reference value $N_{i,0}^x$. The reference values are those yielding $V_m = 0$ V, which is equivalent to a neutral intracellular space $\left(N_{i,0}^{Na^+} + N_{i,0}^{K^+} - N_{i,0}^{Cl^-} - N_{i,0}^{A^-} \right)$. Because N_{A^-} is constant, note that $dN_{A^-} = 0$ in **Equation 15**. Number differences, dN_x , are easier to work with than total numbers of ions N_x (or than concentrations). For typical cell C_m values, voltages in the mV range correspond to net intracellular charge differences (measured as a number of singly charged ions) of the order of attomoles (10^{-18} moles). For instance, when V_m varies in the range [-100mV, 100mV] this corresponds to a change in net singly charged ions of [-20.7, +20.7] amol. With our $Vol_{cell} = 2000 \mu m^3$, this yields tiny concentration changes [-0.01, 0.01] mM. For solutions whose concentrations are in the 1-100 mM range, there could be 4-5 orders of magnitude difference between steady-state values and the changes (in the 0.0001 to 0.01 mM range). Computations based on concentrations, therefore, tend to be unstable. For this reason, calculations in this study are based on changes in ion numbers (in amol units).

Number of intracellular ions

CD models account for the change in intracellular ions at any given moment (Fraser and Huang 2004, 2007; Dijkstra et al 2016), via the following simple relationships of the respective currents:

$$\frac{dN_{Na^+}}{dt} = -\frac{1}{F} \left(I_{Na^+}^T + I_{Na^+}^L + 3I_{pump} \right) \quad (16)$$

$$\frac{dN_{K^+}}{dt} = -\frac{1}{F} \left(I_{K^+}^D + I_{K^+}^L - 2I_{pump} \right) - J_{KCl} \quad (17)$$

$$\frac{dN_{Cl^-}}{dt} = \frac{1}{F} \left(I_{Cl^-}^G + I_{Cl^-}^L \right) - J_{KCl} \quad (18)$$

Setting V_{rest} in SM-CD and other CD models

Experimentally, excitable cells' V_{rest} (i.e., steady-state V_m) values are generally more accessible than cytoplasmic ion concentrations or cell volume, making it useful to anchor a model with a consensus V_{rest} value. For SM-CD, we chose $V_{rest} = -86$, with parameter determinants established in an iterative process as follows: first, a number is chosen for impermeant anions $N_{i,0}^{A-}$, consistent with the system's total cation concentration (as per the extracellular solution). We started with the CN-CD value, knowing that fine-tuning would be needed to meet our (self-imposed) requirement that SM-CD have the same resting Vol_{cell} as CN-CD. Thus, note in **Table 2** the slightly different $N_{i,0}^{A-}$ for CN-CD and SM-CD (likewise, LA-CD and SM-CD, to give identical resting Vol_{cell}).

At rest, Na^+ and K^+ leak currents (for a system with a given pump-strength), must precisely balance. In other words, V_m converges (along with ion concentrations and Vol_{cell}) on steady-state ($=V_{rest}$) when

$$\text{passive } Na^+ \text{ influx} + \text{active } Na^+ \text{ outpump} = \text{zero} \\ \text{AND}$$

$$\text{passive } K^+ \text{ efflux} + \text{active } K^+ \text{ inpump} = \text{zero}.$$

For the $3Na^+/2K^+$ ATPase pump current, this requirement is met when:

$$I_{pump}^{Na^+} = -I_{Na^+}^L = -\frac{3}{2}I_{pump}^{K^+} = -\frac{3}{2}(-I_{K^+}^L) \quad (19)$$

For P-L/D systems with a given pump-strength, V_{rest} varies monotonically with $P_{Na}:P_K$ ratio; low ratios yield hyperpolarized V_{rest} values, high ratios, depolarized ones. $V_{rest} = -86$ mV for SM-CD requires $P_{Na}:P_K = 0.03 : 1$ (likewise for WD-CD). Absolute values for P_{Na} and P_K , and for P_{Cl} (**Table 2**) were guided by the $P_{Na}:P_K:P_{Cl}$ ratio (0.02: 1: 3) reported for amphibian skeletal muscle (Fraser and Huang 2004). For absolute P values, a straightforward constraint was the choice to give SM-CD the same area (C_m) as CN-CD.

As per **Equation 16**, a pump stoichiometry other than $3Na^+out/2K^+in$ would, all else being equal alter V_{rest} . Pump stoichiometry is invariant here, but see Dmitriev et al (2019).

Excitability and safety factor for SM-CD

For SM-CD to be hyperpolarized and appropriately excitable (i.e. “relatively inexcitable”), its input impedance had to be a) notably less than for CN-CD and b) predominantly P_{Cl} -based (Pedersen et al 2016). With resting-P values set, the need to trigger spikes near -60 mV (Fu et al 2011) with a reasonable-sized safety factor (Ruff 2011) had to be met. To achieve safety factor ~ 1.5 , Nav and Kv “densities” in SM-CD were set at 3X the CN-CD level (a larger P_{Cl} would have required even greater V-gated channel densities). Thus, absolute P_{Cl} , P_{Na} and P_K values of SM-CD are biologically appropriate, but leave room for physiological modulation (to, say, alter V_{rest} via ΔP_{Na} or ΔP_K , or to modulate excitability by ΔP_{Cl}).

Table 2 shows that m^3h is vanishingly small at V_{rest} in SM-CD; for CN-CD it adds an extremely small Nav channel contribution to the operational value of P_{Na} that negligibly affects V_{rest} .

Cytoplasmic Donnan effectors

Once V_{rest} is set via P_{Na} and P_K , the intracellular anion concentrations are determined uniquely for the resting state. If Cl^- transport is purely passive (i.e., no involvement of secondary transport) as in SM-CD, then $E_{Cl} = V_{rest}$ and therefore:

$$[Cl^-]_i = [Cl^-]_e \exp\left(\frac{FV_{rest}}{RT}\right). \quad (20)$$

$[A^-]_i$ then follows from the voltage and osmotic balance requirements.

The voltage requirement yields:

$$[Na^+]_i + [K^+]_i - [Cl^-]_i - [A^-]_i = -\Delta c \quad (21)$$

where Δc is the tiny excess concentration of anions associated with V_{rest} and equal to $[intracellular\ anions] - [intracellular\ cations]$:

$$\Delta c = \frac{-V_{rest} C_m}{F Vol_{cell}} \quad (22)$$

$\Delta c = 0.00886$ mM for $V_{rest} = -86$ mV. And the osmotic equilibrium condition is:

$$[Na^+]_i + [K^+]_i + [Cl^-]_i + [A^-]_i = [S]_i = [S]_e \quad (23)$$

where $[S]_i$ and $[S]_e$ are defined below **Equation 13**. **Equations 21** and **23** yield:

$$[A^-]_i = \frac{1}{2} ([S]_i + \Delta c) - [Cl^-]_i \quad (24)$$

A P-L/D model-cell's design for steady-state imposes its $[A^-]_i$. Cell volume adjusts to reflect that constraint $Vol_{cell} = N_i^{A^-} / [A^-]_i$. As mentioned above, SM-CD's $N_i^{A^-}$ was set to give resting $Vol_{cell} = 2000 \mu m^3$, i.e. $N_i^{A^-} = [A^-]_i Vol_{cell}$. Here, the quantity of impermeant anions $N_i^{A^-}$ is invariant, but for many *in vivo* circumstances, it would vary, and variations in Vol_{cell} would be expected in such cases.

Instantaneous perturbations

Experimental solution changes (e.g., as in brain slice experiments) typically require finite "wash-in/wash-out" times. Dijkstra et al (2016) mimicked such solution changes (affecting pump rates and channel gating etc) but here, doing so would have unnecessarily obscured mechanistic underpinnings of responses. Thus, pump-off (anoxia) and pump-on (restoration of pump-strength) changes and channel gating changes (Nav and cation channels open probabilities) are instantaneous.

Maximum cell volume before lysis

Both CN-CD and SM-CD have $C_m = 20$ pF and steady-state $Vol_{cell} = 2000 \mu m^3$. If $0.01 F/m^2 (= 0.01 pF/\mu m^2)$ is the specific capacitance of the bilayer, membrane area is $20/0.01 = 2000 \mu m^2$. With $\frac{4}{3}\pi R^3$ the volume of a spherical cell and $4\pi R^2$ its surface area, maximum Vol_{cell} as the cell swells (to spherical) would be $= 4/3\pi(2000/4\pi)^{3/2} = 8410.4 \mu m^3$. Given a 4% bilayer elasticity strain limit (yielding membrane area = $2080 \mu m^2$), rupture would occur at $8920 \mu m^3$. Thus, in bifurcation plots, the notional Donnan Equilibrium (DE) values indicated for reference, are unachievable by these models. Note too that present models depict neither surface area regulation nor membrane tension homeostasis (see Morris 2018).

Excitatory post-synaptic current (EPSC) via AChR channels

SM-CD action potentials are initiated by EPSCs, i.e. macroscopic end-plate currents through (acetylcholine receptors) AChRs, which are non-selective cation channels that pass Na^+ and K^+ as per the GHK formalism. The EPSC time course mimics the $g(t)$ reported in Wang et al (2004). As reported in Hille (2001), $P_{K^+}^{EPSC} = 1.11 P_{Na^+}^{EPSC}$. The function $g(t)$ has a maximum of 1 and $P_{Na^+}^{EPSC}$ has a 1.5-fold safety factor (i.e., an amplitude adjusted to 1.5X the threshold required to elicit an AP in SM-CD).

The end-plate current is:

$$I_{EPSC}(t) = g(t) (I_{GHK}(Na^+, P_{Na^+}^{EPSC}, V) + I_{GHK}(K^+, P_{K^+}^{EPSC}, V)) \quad (25)$$

Nav-CLS depiction of bleb-damage to Nav-bearing membrane

The extent (in mV) of Nav-CLS as depicted in **Figure 1D** increases with the intensity of bleb-damage (Boucher et al 2012; Morris 2012; Morris et al 2012; Joos et al 2018). Cell survival seems improbable with

100% of the Nav-bearing membrane mechanically bleb-damaged. Arbitrarily, therefore, we model damage to 30% of the Nav population [i.e. **Affected Channels**, AC=0.3]; “damage”, imposed at graded intensities, is “Nav-CLS(0.3) mV”.

The voltage dependences of the rate constants $\alpha_q(V)$ and $\beta_q(V)$ for m and h are the same as in **Equation 7** but shifted by voltage LS (in mV), the **Left Shift**. Therefore, for the Affected Nav Channels (AC), m and h evolve according to:

$$\frac{dq}{dt} = \alpha_q(V + LS)(1 - q) - \beta_q(V + LS)q \quad (26)$$

where q is either m or h .

Computational methods

Calculations involved solving sets of first order differential equations. These were done using Python with the ordinary differential equation solver *odeint*.

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COMPETING INTERESTS

There are no competing interests.

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TABLES

TABLE 1 Chronic Na⁺-overload of DMD patient muscles and *mdx* SMFs

#	REFERENCE	[Na ⁺] _i	NOTES
1	1955 – Horvath et al	[Na ⁺] _i higher (& [K ⁺] _i lower) in dystrophic than in normal muscle fibers	(Horvath et al 1955 is referenced in Rudman et al 1972); biopsy, elemental analysis; fibers of 20 patients with unspecified muscular dystrophies
2	1993 – Dunn et al	diaphragm, gastrocnemius control: 13.0, 13 mdx : 23.5, 24	Two techniques: Na ⁺ -electrode for diaphragm, cyto-volumetrics plus serum and bulk muscle Na values for gastrocnemius. Mice.
3	2008 – Hirn et al	1.4X more ²² Na ⁺ uptake in <i>mdx</i> than in control	²² Na ⁺ uptake. Tetrodotoxin reduces uptake in both and makes them identical (see their Fig 1). Mice.
4	2011 – Miles et al	control: 11.5 mM mdx : 22.5 mM	Na ⁺ -dye measurements. Mice.
5	2011, 2012 – Weber et al	<u>Total sodium content:</u> volunteers: 25-26 mM DMD patients : 38 mM <u>Normalized ²³Na IR (*)</u> volunteers: 0.50 DMD patients : 0.75	²³ Na-MRI. German patient cohort. Hypothesis that DMD edema is an osmotic effect due to ↑ myoplasmic Na ⁺ , not inflammatory extracellular edema as generally thought. (* IR or “inversion recovery”; a means of assessing intracellular Na ⁺ levels)
6	2012 – Lehmann-Horn et al	sustained small decrease of cytoplasmic Na ⁺ (& H ₂ O) overload (n=1)	²³ Na-MRI - pilot study - prolonged “off-label” eplerenone treatment, one 22-yr-old female DMD patient
7	2014 – Altamirano et al	Vastus lateralis control: 8 mM mdx : 18 mM	Na ⁺ -electrodes. Shear-stress stimulation of NO-pathway reduced <i>mdx</i> [Na ⁺] _i to 10 mM without altering control level (see their Fig 1B). Mice.
8	2014 – Burr et al	control: 5.3 mM mdx : 7.3 mM	Na ⁺ -electrodes (see their Fig 10). Mice.
9	2017 – Glemser et al	~20% drop in Na ⁺ & ~15% drop in muscle H ₂ O overloads (n=1)	²³ Na-MRI - pilot study - 6 months “off-label” eplerenone treatment, one 7-yr-old male DMD patient (see their Table 1)
10	2019 – Gerhalter et al	<u>Total sodium content:</u> volunteers: 16.5 mM DMD patients : 26 mM <u>Normalized ²³Na IR</u> volunteers: 0.69 DMD patients : 0.47	²³ Na-MRI - DMD patient cohort in France - authors emphasize that Na ⁺ -loading is regularly observed in patients with DMD even in absence of water T ₂ increases (see also Gerhalter et al 2017)

Table 2 – Parameter values for CN-CD and SM-CD models and variants.

CN-CD entries are from Table 1 of Dijkstra et al (2016). For SM-CD values and other models, see Methods and text. When terms are listed two ways (e.g. C { C_m }) the first is carried over from Dijkstra et al (2016) for use in equations but is usually referred to here (in text and figures) in the second way. All permeabilities (in units of $\mu\text{m}^3/\text{s}$) are specific to the invariant cell area used here, as per Dijkstra et al (2016). (Constants: F ; Faraday constant: 96485.333 C/mol; R , universal gas constant, 8.3144 J/K; T , Absolute temperature, 310 K; Specific membrane capacitance, 0.01 pF/ μm^2 .)

Cell characteristics		CN-CD	MN-CD	SM-CD	WD-CD	LA-CD
C { C_m }	Membrane capacitance	20 pF	20 pF	20 pF	20 pF	20 pF
P_{Na}^T	Maximal transient Na^+ permeability {“Nav channels”; akin to $g_{\text{Na}_{\text{max}}}$ of HH}	800 $\mu\text{m}^3/\text{s}$	800 $\mu\text{m}^3/\text{s}$	2400 $\mu\text{m}^3/\text{s}$	2400 $\mu\text{m}^3/\text{s}$	2400 $\mu\text{m}^3/\text{s}$
P_{K}^D	Maximal delayed rectifier K^+ permeability {“Kv channels”; akin to $g_{\text{K}_{\text{max}}}$ of HH}	400 $\mu\text{m}^3/\text{s}$	400 $\mu\text{m}^3/\text{s}$	1200 $\mu\text{m}^3/\text{s}$	1200 $\mu\text{m}^3/\text{s}$	1200 $\mu\text{m}^3/\text{s}$
P_{Na}^L { P_{Na} }	Na^+ leak permeability	2 $\mu\text{m}^3/\text{s}$	2 $\mu\text{m}^3/\text{s}$	0.3 $\mu\text{m}^3/\text{s}$	1.06 $\mu\text{m}^3/\text{s}$	0.3 $\mu\text{m}^3/\text{s}$
P_{K}^L { P_K }	K^+ leak permeability	20 $\mu\text{m}^3/\text{s}$	20 $\mu\text{m}^3/\text{s}$	10 $\mu\text{m}^3/\text{s}$	36.7 $\mu\text{m}^3/\text{s}$	10 $\mu\text{m}^3/\text{s}$
P_{Cl}^L { P_{Cl} }	Cl^- leak permeability	2.5 $\mu\text{m}^3/\text{s}$	2.5 $\mu\text{m}^3/\text{s}$	30 $\mu\text{m}^3/\text{s}$	2.5 $\mu\text{m}^3/\text{s}$	30 $\mu\text{m}^3/\text{s}$
$P_{Na}:P_K:P_{Cl}$	Ratios of leak permeabilities	0.1: 1: 0.125	0.1: 1: 0.125	0.03: 1: 3	0.03: 1: 0.07	0.03: 1: 3
$I_{\text{max pump}}$	Maximal electrogenic I_{pump} of $3\text{Na}^+/2\text{K}^+$ ATPase {“ Q_{pump} ” in Dijkstra et al. (2016) }	54.5 pA	54.5 pA	54.5 pA	54.5 pA	16.35 pA
Pump-strength	max ATP consumption rate	566 amol/s	566 amol/s	566 amol/s	566 amol/s	170 amol/s
U_{KCl}	K^+/Cl^- co-transporter strength	1.3 fmol/(V s)	0	0	0	0
P_{Cl}^G	Maximal voltage-dependent Cl^- permeability {“ $g_{\text{Cl}}(V)$ ”}	19.5 $\mu\text{m}^3/\text{s}$	19.5 $\mu\text{m}^3/\text{s}$	0	0	0
L_{H_2O}	Effective cell water permeability	2 $\mu\text{m}^3/(\text{s Pa})$	2 $\mu\text{m}^3/(\text{s Pa})$	2 $\mu\text{m}^3/(\text{s Pa})$	2 $\mu\text{m}^3/(\text{s Pa})$	2 $\mu\text{m}^3/(\text{s Pa})$
N_i^A	Intracellular impermeant anion quantity	296 fmol	296 fmol	299.2 fmol	299.2 fmol	298.4 fmol
Fixed extracellular osmolytes						
$[\text{Na}^+]_e$	Extracellular Na^+ concentration	152 mM	152 mM	152 mM	152 mM	152 mM
$[\text{K}^+]_e$	Extracellular K^+ concentration	3 mM	3 mM	3 mM	3 mM	3 mM
$[\text{Cl}^-]_e$	Extracellular Cl^- concentration	135 mM	135 mM	135 mM	135 mM	135 mM
$[\text{A}^-]_e$	Extracellular impermeant anion concentration	20 mM	20 mM	20 mM	20 mM	20 mM

... cont'd Table 2

Cell characteristics		CN-CD	MN-CD	SM-CD	WD-CD	LA-CD
[S] _e	Total extracellular solute concentration	310 mM	310 mM	310 mM	310 mM	310 mM
Variable quantities (values are those for "resting" or steady-states, such as V_{rest})						
[S] _i	Total intracellular solute concentration	310 mM	310 mM	310 mM	310 mM	310 mM
$V\{V_m\}$	Membrane potential	-65.5 mV	-64.3 mV	-86.0 mV	-86.0 mV	-85.6 mV
[Na ⁺] _i	Intracellular Na ⁺ concentration	10 mM	9.84 mM	3.7 mM	6.8 mM	6.54 mM
[K ⁺] _i	Intracellular K ⁺ concentration	145 mM	145.2 mM	151.3 mM	148.2 mM	148.5 mM
[Cl ⁻] _i	Intracellular Cl ⁻ concentration	7 mM	12.2 mM	5.4 mM	5.4 mM	5.5 mM
[A ⁻] _i	Intracellular impermeant anion concentration	148 mM	142.8 mM	149.6 mM	149.6 mM	149.5 mM
E_{Na^+}	Na ⁺ Nernst potential	72.6 mV	73.1 mV	99.2 mV	82.9 mV	84.0 mV
E_{K^+}	K ⁺ Nernst potential	-103.6 mV	-103.6 mV	-104.7 mV	-104.2 mV	-104.2 mV
E_{Cl^-}	Cl ⁻ Nernst potential	-79.0 mV	-64.3 mV	-86.0 mV	-86.0 mV	-85.6 mV
$I_{Na^+}^L \{I_{Na^+}^{leak}\}$	Steady state Na ⁺ leak current	-78.2 pA	-77.1 pA	-14.75 pA	-52.1 pA	-14.66 pA
$I_{Na^+}^T$	Steady state Na ⁺ window current	-0.07 pA	-0.14 pA	-2.3x10 ⁻⁷ pA	-2.4x10 ⁻⁷ pA	-3.3x10 ⁻⁷ pA
$W\{Vol_{cell}\}$	Cell volume	2000 μm ³	2072 μm ³	2000 μm ³	2000 μm ³	2000 μm ³
m	Nav activation gate	0.013	0.013	0.000	0.000	0.000
h	Nav inactivation gate	0.987	0.987	1.000	1.000	1.000
m^3h	Nav open probability	2.2 x 10 ⁻⁶	2.2 x 10 ⁻⁶	1.75 x 10 ⁻¹²	1.000	2.78 x 10 ⁻¹²
n	K ⁺ delayed rectifier (Kv) gate	0.003	0.003	0.000	2.0 x 10 ⁻¹²	2.78 x 10 ⁻¹²
ATP/s	Steady-state ATP consumption	270.9 amol/s	267.3 amol/s	51.1 amol/s	180.4 amol/s	50.7 amol/s
Max ATP/s	Max ATP consumption rate	566 amol/s	566 amol/s	566 amol/s	566 amol/s	170 amol/s
Pump-reserve	Max / Steady-state ATP consumption	2.09	2.12	11.08	3.14	3.35
Miscellaneous						
Membrane area	Surface Area of cell based on 20 pF capacitance	2000 μm ²	2000 μm ²	2000 μm ²	2000 μm ²	2000 μm ²
W_{Donnan}	"cell" volume $\{Vol_{cell}\}$ at Donnan Eq (if bilayer indefinitely expansible)	14,800 μm ³	14,800 μm ³	14,960 μm ³	14,961 μm ³	14,952 μm ³
$W_{rupture}$	Approx. rupture Volume for Surface Area of 20 pF (4% max bilayer expansion)	8920 μm ³	8920 μm ³	8920 μm ³	8920 μm ³	8920 μm ³

TABLE 3
Voltage-dependent rates for H-H gating variables *

Term	Expression	Description
$\alpha_m(V)$	$\frac{0.32(V + 52 \text{ mV})}{1 - \exp\left(-\frac{V + 52 \text{ mV}}{4 \text{ mV}}\right)} \frac{\text{kHz}}{\text{mV}}$	Activation rate for transient Na ⁺ channel
$\beta_m(V)$	$\frac{0.28(V + 25 \text{ mV})}{\exp\left(-\frac{V + 25 \text{ mV}}{5 \text{ mV}}\right) - 1} \frac{\text{kHz}}{\text{mV}}$	Deactivation rate for transient Na ⁺ channel
$\alpha_h(V)$	$0.128 \exp\left(-\frac{V + 53 \text{ mV}}{18 \text{ mV}}\right) \text{ kHz}$	Inactivation rate for transient Na ⁺ channel
$\beta_h(V)$	$\frac{4}{1 + \exp\left(-\frac{V + 30 \text{ mV}}{5 \text{ mV}}\right)} \text{ kHz}$	Recovery from inactivation rate for transient Na ⁺ channel
$\alpha_n(V)$	$\frac{0.016(V + 35 \text{ mV})}{1 - \exp\left(-\frac{V + 35 \text{ mV}}{5 \text{ mV}}\right)} \frac{\text{kHz}}{\text{mV}}$	Activation rate for delayed rectifier K ⁺ channel
$\beta_n(V)$	$0.25 \exp\left(-\frac{V + 50 \text{ mV}}{40 \text{ mV}}\right) \text{ kHz}$	Deactivation rate for delayed rectifier K ⁺ channel

* Based on Kager et al (2000) but as used in Dijkstra et al (2016) with one typographical error corrected.

FIGURES

Figure 1

Dystrophic muscle fibers, ischemia and bleb-damaged sarcolemma

A. SMF ischemia in DMD has several distinct causes **1.** Vascular insufficiency (Verma et al 2019; Podkalicka et al 2019). **2.** Malfunctioning pumps in bleb-damaged sarcolemma that accumulates because DMD fibers' regenerative capabilities are impaired (Chang et al 2016; Shattock & Matsuura 1993; Williams and Bloch, 1999; Whitehead et al 2010; Juel et al 2015; Lansman 2015; Burr and Molkentin 2015; McElhanon & Bhattacharya 2018; Murphy et al 2019). **3.** Exertion-induced functional ischemia (Sander et al 2000; Allen et al 2016). **B.** Excessive fiber-isolation-induced bleb-damage to fragile (dystrophin-minus) Nav-bearing sarcolemma of *mdx* fibers would explain their "leaky" Nav1.4 channels (Hirn et al 2008; see Morris, 2018). **C.** Depiction of

García-Pelagio et al (2011) pipette aspiration tests that quantified *mdx* fiber susceptibility to bleb-damage; bleb-damage signifies loss of non-covalent binding (adhesion) between bilayer and membrane skeleton proteins (e.g., F-actin) that fosters, e.g., decay of cell-mediated structural features of the bilayer (Methfessel et al 1986; Sheetz et al 2006; Phillips et al 2009; Lundbaek et al 2004, 2010; Morris 2012; Ameziane-Le Hir et al 2014; Cornelius et al 2015; Petrov et al 2017; Burden et al 2018). That in turn alters conformational stabilities of membrane proteins (Morris and Horn 1991; Morris et al 2012A,B; Zhang et al 2000; Zhang and Hamill 2000; Burr and Molkentin, 2015). Rectangles signify ion transporting proteins that malfunction in damaged sarcolemma (e.g., Na^+/K^+ -pumps, Na^+ -permeant channels). **D.** For the computational Nav channel used here, window conductance ($\text{m}^3\text{h}(\text{V}_m)$) before and after a 20 mV Nav-CLS or Nav Coupled-Left-Shift. Explanation: SMF-Nav and neuronal-Nav channels undergo irreversible hyperpolarizing shifts in bleb-damaged membrane (Boucher et al 2012; Morris and Joos 2016; Joos et al 2016). In Hodgkin-Huxley (H-H) terms, fast activation (m^3) and inactivation (h) both shift "leftward" by the same amount ("CLS").

Figure 2 Anoxic rundown and recovery

Ai. anoxic rundown of SM-CD (experimental equivalent: instantaneous exposure to pump-inhibitor, ouabain). Following the small pump-off depolarization, rundown is limited by [small $I_{Na\text{leak}}$]. Since $P_K \gg P_{Na}$, concurrent K^+ efflux can almost maintain cytoplasmic electroneutrality. With P_{Cl} so large, ΔE_{Cl} tracks ΔV_m , minimizing the driving force on Cl^- ; rundown-swelling is minor because, with $\Delta[Na^+]_i$ rate-limiting, $\uparrow[Na^+ + Cl^-]_i$ is minor. Finally, 22.7 minutes into anoxia, SM-CD reaches the Na_v channel activation threshold; I_{Na} from “myogenic” APs elicits a catastrophic concurrent $[Cl^- + H_2O]$ influx. SM-CD swells and degrades toward DE. **ii, iii, iv,** SM-CD $V_m(t)$ before/during/after anoxia is imposed, with 100% pump-strength restored at different times, as labeled. **Bi.** In SM-CD a $\downarrow P_{Na}$ (as labeled and explained in the text) prolongs the anoxic rundown time to ectopic firing, whose start and termination is expanded in **ii, iii.** **Ci,** anoxic rundown of LA-CD (as per **Table 2**, steady-state Vol_{cell} for LA-CD and SM-CD are identical because the impermeant cytoplasmic anion quantity in LA-CD was slightly decreased). **ii,** ion currents in the minutes before/during/after spontaneous myogenic firing (the inset box’s greatly expanded vertical resolution emphasizes the rundown currents before firing), **iii,** restoring LA-CD pump-strength (30%) during myogenic firing (top) and just after it ceases (bottom).

Figure 3 SM-CD steady-state bifurcation plots

A. The SM-CD bifurcation plot for steady-state V_m as a function of pump-strength; the steady-state or stable values form continua; unstable values occur at points # and the saddle-node, X; DE is the Donnan Equilibrium point). This illustrates what it means to say that steady-state P-L/D values vary non-linearly (and bistably) as pump-strength varies (see Joos and Morris, submitted); for DMD fibers, the ischemic "danger zone" (boxed region), which includes the saddle-node (X), is critical. **B.** Ischemic range for steady-state $[Na^+]_i$ up to the unstable saddle node. **C.** Ischemic region for each of the SM-CD parameters, as labelled. Approaching the saddle-node, i.e., in the danger zone, the system shows a steep $\downarrow$ ATP-consumption ($=\downarrow$ hyperpolarizing I_{pump}) concomitant with a steep $\uparrow$ $[Na^+]_i$ (~ 10 mM to ~ 90 mM). This occurs because from 10-90 mM, pump-rate sensitivity to $[Na^+]_i$ (see **Fig methpump**) is radically shallower than close to normal $[Na^+]_i$ (i.e. up to ~ 10 mM). In the danger zone, an I_{pump} value sufficient to achieve steady-state requires a large $[Na^+]_i$, but this entails E_{Na} depletion and concomitant E_K depletion. The resultant depolarization further diminishes I_{NaLeak} ; hence the $\downarrow$ ATP-consumption (at steady-state, recall, $-I_{NaLeak} = \text{hyperpolarizing } I_{pump}$). Finally, when pump-strength is so low that pump-reserve ≤ 1 , the excitable P-L/D system is depolarized to X, i.e. to its unstable saddle-node.

Figure 4
Bleb-damage induced
Nav-CLS in CN-CD and
SM-CD

For CN-CD (A) and SM-CD (B), 30% (affected channels, i.e., $AC = 0.3$) of the Nav-bearing membrane is damaged at Nav-CLS intensities ranging from 0 mV (magenta and green dots = healthy steady-state) up to 10 mV for CN-CD or up to 30 mV for SM-CD. Most (70%) of the Nav channels are as depicted in Figure 3B but 30% (the Navs in bleb-damaged membrane), having undergone Nav-CLS, have their m^3 and h shifted by the

indicated amount (the non-dimensional “window conductance” consequence of $\text{NavCLS} = 20 \text{ mV}$ is illustrated in Figure 1D). Bii, for SM-CD, $V_m(t)$ as labeled. A and B, top panels reveal subthreshold Nav-CLS effects; negative slopes indicate increasing hypersensitivity to a test stimulus. Vertical blue lines indicate where spontaneous “ectopic” (or myogenic for SMFs) firing begins. Beyond that, CN-CD’s small pump-reserve cannot counteract the Na^+ -loading; $[Na^+ + Cl^- + H_2O]_i$ (thence Vol_{cell}) increases catastrophically (=cytotoxic swelling). This accords with excitation-related Na^+ -overload computations of Dijkstra et al (2016) but shows more biophysical nuance because Nav-CLS intensity is graded and Nav-CLS onset is instantaneous (to mimic excitotoxic veratridine, Dijkstra et al set the H-H parameter, h , to 1, and provided a “wash-in” period). Its strongly hyperpolarized V_{rest} lets SM-CD withstand more intense Nav-CLS damage (than CN-CD) before firing ectopically. Thanks to SM-CD’s big pump-reserve (pump-strength= 100% here), firing, when first it develops, does not elicit cytotoxic swelling.). At 28 mV it causes somewhat irregular 5-6 Hz tonic firing and 28.5 it causes bursts of 16-18 APs, with intra-burst frequency ~25 Hz. Only at Nav-CLS=30 mV, does SM-CD succumb to swelling. The Cl^- -entry plot (not shown) has the same shape as $Vol_{cell}(\text{Nav-CLS})$. Beyond the blue line (Nav-CLS-induced myogenic firing), SM-CD’s complex parameter changes reflect P-L/D feedback plus the changing excitation patterns. As per Boucher et al (2012), tonic firing gives way to bursts (Bii) then (not shown) to depolarizing block. At Nav-CLS=28 mV and 28.5 mV, a ~3-fold $\uparrow$ ATP-consumption prevents major Na^+ -loading ($[Na^+]_i$ reaches only ~6 mM). Swelling is minimal. At Nav-CLS=~29 mV, I_{pump} hyperpolarizes V_{rest} . At 30 mV, with $(\text{hyperpolarizing } I_{pump}) < (\text{depolarizing } (I_{Na\text{leak}} + \text{time-averaged } I_{Nav}))$, the P-L/D system is overwhelmed.

Figure 5

Leaky cation channels

For three minimal P-L/D models (Donnan dominated SM-CD and LA-CD, and Pump-Leak dominated WD-CD), the P-L/D processes are plotted during an identical 4 min step-activation (then step-deactivation) of cation channels (see **Equation 4**). (The on/off would apply if, as suggested by (Lansman 2015), these were mechanosensitive channels rendered hyper-mechanosensitive (Vandorpe et al 1994) in DMD-damaged sarcolemma, experiencing transient localized membrane stretch. If, more simply, damage induced an open probability increase, there would be an on-step only.) For SM-CD and LA-CD, this increases "resting" P_{Na} by 42% and for WD-CD, by 12%. The $V_m(t)$ step-on/step-off jumps are "RC" relaxations. For all 3 cases V_{rest} is far from E_{Na} , near E_K , and $E_{Cl}=V_{\text{rest}}$. The depolarizing/hyperpolarizing jumps therefore mostly reflect the onset/offset of inward I_{Na} . Slow $V_m(t)$ changes reflect the active (see ATP-consumption) and passive ion homeostatic feedback fluxes as each model moves toward a new steady-state. Swelling rates are determined by the smaller contributor to net osmotic influx, $[\text{Na}^+ + \text{Cl}^-]$. Since driving forces on Cl^- are very small, Vol_{cell} changes lag the $[\text{Na}^+]_i$ changes even in [big P_{Cl}] SM-CD and LA-CD. In Pump-Leak dominated ("neuron-like") WD-CD, Vol_{cell} changes very little and very slowly due to [small P_{Cl}] plus the small Cl^- driving force. Cl^- influxes, not shown, scale exactly with Vol_{cell} changes.

A though chronically-ischemic (pump-strength 30%)
LA-CD handles a physiological stressor
 (EPSC-triggered APs at 120 Hz for 10 s)

B i the physiological stressor *plus* both pathological insults
 of the dystrophin-minus (DMD) condition:
 “too little Na^+ -pumping” (pump-strength at 30%) &
 “too much Na^+ -leak” (bleb-damage induced leaks)

Figure 6

Stress-testing the ischemic model, LA-CD, without/with leaky channels

A, V_m for LA-CD before/during/after the described stress-test. **Bi**, V_m for LA-CD with “bled-damage” (and the resulting Na^+ -leaks as described) vs LA-CD before/during/after the same stress-test. **ii**, as the stress-test ends at 10 s, expanded view of the transition from stimulated to ectopic (“myogenic”) APs.

PUMP-LEAK/DONNAN

MINIMAL P-L/Donnan ion homeostasis systems at steady-state

- no net cation fluxes (precise pump-leak counterbalance)
- no net anion flux (Donnan effectors enable Nernst balance)
- $V_{rest} = E_{Cl}$
- each compartment is electroneutral and water activity in each is the same (no osmotic pressure)

DONNAN DOMINATED P-L/D [big P_{Cl}][small I_{NaLeak}]

PUMP-LEAK DOMINATED P-L/D [small P_{Cl}][big I_{NaLeak}]

Figure 8

Pump-Leak/Donnan ion homeostasis: two ends of the spectrum

Cytoplasm's impermeant anions (mostly proteins) are Donnan effectors whose potentially lethal cell-swelling forces are integral to animal cell ion homeostasis, which is a Pump-Leak/Donnan (P-L/D) process. P-L/D processes autonomously maintain and restore (post-ionic perturbations) steady-state $[ion]_{i(ntracellular)}$, cell volume (Vol_{cell}) and membrane potential (V_m). In a minimal P-L/D system, steady-state $V_m(=V_{rest})$ depends on $P_{Na}:P_K$ and the electrogenicity and $[Na^+]_i$ -sensitivity of the pump, $3Na^+/2K^+$ -ATPase. The membrane is H_2O -permeable and, via P_{Cl} , permeable to small anions (here, just Cl^-). Steady-state Vol_{cell} depends on the molar quantity (not the concentration) of Donnan effectors. SM-CD (the model used here for to represent the P-L/D processes of SMFs) is a minimal P-L/D system to which excitability-machinery is added. During restoration to steady-state following any perturbation, the autonomous P-L/D process can be

summarized as follows: 1. the ATP-consuming electrogenic pump "listens" and responds (only) to $[Na^+]_i$, while 2. Passive ion fluxes via P_{Cl} , P_{Na} , P_K "feel" and respond to their electrodiffusive driving forces while standard thermodynamics ensures that no inside/outside H_2O -activity differential develops. Thus, cytoplasmic electroneutrality and transmembrane osmo-balance are maintained even as the pump ($3Na^+$ -out/ $2K^+$ -in; net effect, a hyperpolarizing I_{Na}) works, rebuilding cellular ion-batteries. Thus, at steady-state in SM-CD, the small hyperpolarizing $I_{Na,pump}$ =the small depolarizing I_{NaLeak} . These cartoons depict the minimum elements needed for P-L/D steady-states dominated by anion fluxes or cation fluxes, as previously (Joos & Morris, submitted) explained. Whereas SM-CD is a minimal Donnan dominated P-L/D system, CN-CD is a **non**-minimal Pump-Leak dominated P-L/D system (a K/Cl co-transporter puts its E_{Cl} negative to V_{rest} ; see footnote, **Table 2**). The Nav and Kv channels (whose m^{3h} and n values at V_{rest} are negligible) and the ACh-activated cation channels (AChRs) underlying excitatory synaptic current-triggered APs do not contribute to steady-state, so are not depicted.

Figure 9

ATP-consumption and $[Na^+]_i$

A. For all the CD models here, a stand-alone biochemical descriptor of the pump model (Hamada et al, 2003) i.e., a $3Na^+/2K^+$ -ATPase kinetics plot (substrate: Na^+_i) (see **Equation 10**). The CD models assume a fixed external milieu, so the pump is insensitive to $[K^+]_e$. Steady-state values for SM-CD and CN-CD are indicated. The right axis shows the equivalent hyperpolarizing electrogenic I_{pump} (maximally, 54.5 pA). **Bi.** The standard (100%) curve is multiplied by scaling factors to depict ischemia (pump-strengths <100%) and up-regulation (pump-strengths >100%) with logarithmic Y-axes. The dot-dash green line is SM-CD's resting ATP-consumption. **Bii.** expanded lower left sector to show that, notwithstanding almost-identical steady-state ATP-consumption, SM-

CD (100% pump-strength) and LA-CD (30% pump-strength) achieve very different $[Na^+]_i$ thanks to steep kinetics in the substrate's normal physiological range.