The Pump-Leak/Donnan ion homeostasis strategies of skeletal muscle fibers and neurons

Béla Joos^{1,2} and Catherine E Morris^{2,3}

¹Department of Physics, University of Ottawa, 150 Louis Pasteur Priv., Ottawa, Ontario, Canada
K1N 6N5

²Center for Neural Dynamics, University of Ottawa, Ottawa, Ontario, Canada

³Neuroscience, Ottawa Hospital Research Institute, 451 Smyth Road, Ottawa, Ontario, Canada,
K1H-8M5

ABSTRACT

Skeletal muscle fibers (SMFs) and neurons are low and high duty-cycle excitable cells constituting exceptionally large and extraordinarily small fractions of vertebrate bodies. The immense ClC-1-based chloride-permeability (P_{Cl}) of SMFs has thwarted understanding of their Pump-Leak/Donnan (P-L/D) ion homeostasis. After formally defining P-L/D set-points and feedbacks, we therefore devise a simple yet demonstrably realistic model for SMFs. Hyper-stimulated, it approximates rodent fibers' ouabain-sensitive ATP-consumption. Size-matched neuron-model/SMF-model comparisons reveal steady-states occupying two ends of an energetics/resilience P-L/D continuum. Excitable neurons' costly vulnerable process is Pump-Leak dominated. Electrically-reluctant SMFs' robust low-cost process is Donnan dominated: collaboratively, Donnan effectors and [big P_{Cl}] stabilize V_{rest} , while SMFs' exquisitely small P_{Na} minimizes ATP-consumption, thus maximizing resilience. "Classic" excitable cell homeostasis ([small P_{Cl}][big I_{NaLeak}]), *de rigueur* for electrically-agile neurons, is untenable for vertebrates' (including humans') major tissue. Vertebrate bodies evolved thanks to syncytially-efficient SMFs using a Donnan dominated ([big P_{Cl}][small I_{NaLeak}]) ion homeostatic strategy.

INTRODUCTION

Ion Homeostasis is a Pump-Leak/Donnan (P-L/D) feedback process

Ion homeostasis is the energy-consuming feedback process by which a cell maintains its steady-state volume (Vol_{cell}), membrane potential (V_m) and ion concentrations ($[ion]_i$), returning autonomously to steady-state after ionic perturbations by sensing a change and transiently adjusting its fluxes and energy consumption till steady-state is restored. In the classic feedback control situation, thermostatic regulation of room temperature, a temperature sensor/effector system alters the output of a heat source in proportion to deviations from a chosen temperature set point. As per **Figure 1A** and legend, ion homeostatic feedback too, has sensor/effector elements and a set point. For any particular cell-type, the ion homeostasis set point is a steady-state collection -- $\{Vol_{cell}, V_m, [ion]_i\}$ -- "chosen" by evolution. Evolution furnishes that cell-type with: a particular quantity (Usher-Smith et al, 2009) of anionic Donnan effectors, an H_2O -preamble membrane of a particular area (C_m) equipped with particular densities of Na^+ , K^+ and Cl^- permeant pathways (P_{Na} , P_K , P_{Cl}), and electrogenic ($3Na^+/2K^+$ -ATPase) pumps sufficient to sustain steady-state and restore it after perturbations of the size expected for that cell-type. Quantitative cell-type specific differences for these components account for different ion homeostatic set points. The minimal components for self-regulating Pump-Leak/Donnan (P-L/D) ion homeostasis are depicted in **Figure 1B**. In virtually all cells, ion homeostasis will be non-minimal since most deploy additional ion transporters and channels to address nuanced physiological requirements (Fraser and Huang 2004), but these components tune rather than cause P-L/D ion homeostasis and so are addressed here only peripherally.

Evolutionary context for muscle and nerve ion homeostatic strategies

Skeletal muscle is the human body's biggest tissue (~40%; Janssen et al 2000). This tissue's fundamental ion homeostatic mechanisms evolved to meet the needs of our early vertebrate ancestors. Their bodies comprised, perhaps, 40-60% skeletal muscle fiber (SMF) (see **Table 1**). Then, as now, SMFs would have been low duty-cycle excitable cells (i.e., at V_{rest} except during brief, infrequent action potentials (APs)). Also then as now, vertebrate neurons would have been high duty-cycle electrically-agile input-summing cells tasked by the whole-organism with overseeing sensory processing and controlling the timing of muscle contractions. For those ancestral vertebrates, a low-cost SMF resting-state would

have been an enormous boon, while, given the tiny body portion devoted to brain in early vertebrates (**Table 1**), heavy ion homeostatic spending on neurons would have been even more tolerable than (say) in modern mammals. That ancient tissue-mass difference plus the starkly different electrophysiological lifestyles of skeletal muscle and neurons likely governed the evolution of the distinctive ion homeostatic strategies we identify here as operative in modern mammals (and modern amphibians, etc).

Ion homeostasis modeling via the charge difference approach

To explain the self-restoring V_{rest} of frog SMFs and the Na^+ -transporter regulation of SMF volume, Fraser and Huang (2004) introduced a charge difference (CD) approach. It explicitly includes Donnan effectors and counts all transmembrane ion and H_2O movements. This enables simultaneous computation of steady-state Vol_{cell} , $[ion]_i$ and V_m (via charge on C_m) (Fraser and Huang 2007, Usher-smith et al 2009; Kay 2018; Dmitriev et al 2019). Where relevant issues warrant it, CD-model trajectories of perturbed (non-steady-state) systems can be examined on timescales from milliseconds to days. Fraser and Huang's (2004) seminal work described non-excitable SMFs. A later elaboration addresses APs and ion homeostasis in t-tubules (Fraser et al 2011). SM-CD, the excitable SMF model devised is as simple as an autonomous ion homeostasis model can be. It depicts not an entire multinucleate SMF but, to enable direct comparison with CN-CD, an existing (Dijkstra et al 2016) CD model for cortical neurons (**Figure 8 A,B,C**) an iterable fraction of a SMF (**Tables 1 and 2, Figure 8C,D**).

CD modeling comparisons here highlight two overlooked facets of SMF physiology: **1)** the collaboration of CIC-1 channels with Donnan effectors is imperative for SMF ion homeostasis (not just for low SMF excitability (Jentsch and Pusch, 2018; Jeng et al 2020) and **2)** the extraordinary smallness of the (unidentified) SMF P_{Na} renders it pivotal (via its [small I_{Naleak}]) to the robustness of SMF ion homeostasis.

Donnan forces finessed as “found free-energy” for volume homeostasis

Though ion homeostasis is commonly referred to as a Pump-Leak process, dropping “Donnan” abrogates the P-L/D process; the Donnan-mediated volume feature is mandatory. **Figure 1B** (and legend) introduces the simple but novel idea that at steady-state, P-L/D ion homeostasis can be Donnan dominated or Pump-Leak dominated. Each of the two systems depicted has the minimal components required for autonomous P-L/D ion homeostasis; they differ in relative densities of always-open (“resting” or “leak”) channels, P_{Cl} , P_K and P_{Na} .

At steady-state in the Donnan dominated system, the transmembrane flux of ions is due mostly to Cl^- ions, with influx and efflux perfectly balanced thanks to the cytoplasm's impermeant anions, while at steady-state in the Pump-Leak dominated system, the transmembrane ion flux is due mostly to cations, with passive Na^+ -influx and K^+ -efflux perfectly balanced by active (pumped) counterfluxes. Animal cells' ever-present Donnan forces are seen as a potentially lethal problem for cells to solve. When pumps falter, after all, $[Na^+ + Cl^- + H_2O]$ entry kills cells as a direct consequence of Donnan forces. Animal cells handle the $[Na^+ + Cl^- + H_2O]$ -danger by sequestering the perilous free energy. Neurons use [small P_{Cl}], SMFs use [small P_{Na}].

Neuronal steady-state is thus a [small P_{Cl}][big I_{Naleak}] Pump-Leak dominated process while SMF steady-state is a [big P_{Cl}][small I_{Naleak}] Donnan dominated process. Till now however, the distinction between Donnan dominated and Pump-Leak dominated strategies has, however, gone unrecognized (or at least unnamed). CIC-1 channels, expressed abundantly and exclusively in SMFs, are the major component (Pedersen et al 2016) of their [big P_{Cl}]. Discovered in 1991 by the Jentsch lab (Steinmeyer et al 1991) via close homology to elasmobranch CIC-0 (see Miller 2015), their role is described in

electrophysiological terms; i.e, CIC-1 passively stabilizes the hyperpolarized V_{rest} (Jentsch & Pusch 2018). The P-L/D framework shows that, simultaneously, CIC-1 is crucial for SMF ion homeostasis. A counterfactual model (a big P_K SMF-analog P-L/D) helps clarify this point.

Cytoplasmic proteins did not evolve to serve ion homeostasis. Nevertheless the unavoidable “colligative-charge” attribute that makes them Donnan effectors is a source of “found free energy”, one that cells use in a sensor/effector role for ion homeostatic feedback (**Figure 1A**). Low-excitability SMFs evolved finessing their Donnan effector/sensor system, making it a high-conductance (=low-impedance) stabilizer of their low-cost ion homeostatic steady-state ($E_{Cl}=V_{rest}$). Modeling here reveals how, by maintaining [small I_{NaLeak}] and exploiting that found free energy, SMFs obtain superb operational resilience. Donnan dominated SMF ion homeostasis, we propose, played an important role in the vertebrate lineage. Our initial motivation was, however, to understand SMF longevity in Duchenne muscular dystrophy (DMD) patients (Allen et al 2016; Gerhalter et al 2019) where SMFs experience chronic ion homeostatic stresses that rapidly kill neurons. In a companion paper (Morris et al submitted), SM-CD, the P-L/D model for SMF ion homeostasis explained here, is used to address that issue.

RESULTS

SM-CD passes a stress-test

How SM-CD was devised is explained in **Methods**. Because SM-CD is new, we begin by demonstrating its general merit via a “reality check” (**Figure 2A**). Clausen (2015) summarized experimental findings (Nielsen and Clausen 1997) as follows: “In isolated rat soleus... after 10 sec of stimulation at 120 Hz, the net Na^+ re-extrusion measured in the subsequent 30 sec of rest reaches a 22-fold increase in Na^+ , K^+ pump activity corresponding to 97% of the theoretical maximum rate of active Na^+ , K^+ pumping measured and calculated on the basis of total content of 3H -ouabain binding sites”. **Figure 2A** follows V_m , $[Na^+]_i$, $[Cl^-]_i$, Vol_{cell} and ATP-consumption in SM-CD while 1200 APs fire and the while SM-CD fully restores itself to steady-state. The “ Na^+ re-extrusion” time course is reassuringly similar to rat soleus. In SM-CD, the maximal $\uparrow I_{pump}$ is 11.1-fold. When increased to 22.2-fold (**Figure Sup1A**) ATP-consumption(t) responds accordingly. **Figure 2B** shows one EPSC-elicited AP in SM-CD and the trajectories (milliseconds, minutes) of its ion homeostatic parameters. Having demonstrated SM-CD’s response to challenge, we compare neuron versus SMF P-L/D processes. The biophysics of single AP ion homeostasis will be addressed later (**Figure 6**).

Steady-state, anoxic rundown, recovery: P-L/D systems without excitability

If their sole energy-generator (hyperpolarizing I_{pump}) is eliminated, P-L/D systems degrade towards Donnan equilibrium (DE). Dijkstra et al (2016) mimicked brain-slice anoxia in CN-CD, making switch-off gradual (like wash-in of pump-inhibitors). Here, for biophysical clarity, “pump-off/on” is instantaneous.

In **Figure 3**, to examine steady-state P-L/D components only, all V-gated channels are zeroed (*). For CN- or CN*-CD and for SM- or SM*-CD, steady-states are identical. Before $t=0$, currents=zero and Vol_{cell} , V_{rest} , $[ion]_i$ and ATP-consumption are as per CN-CD, SM-CD, **Table 2**. At pump-off ($t=0$), rundown commences. Pumping is restored at 105 minutes (before swelling to rupture point, and long before “notional” DE; see **Table 2**, Miscellaneous).

Before $t=0$, steady-state ATP-consumption already tells a major story: CN*-CD maintains $V_{rest}=-65.5$ mV for 270.9 amol/s, whereas SM*-CD maintain $V_{rest}=-86$ mV for 51.1 amol/s.

The frugality of SM*-CD reflects the fact that ATP-consumption directly mirrors I_{Naleak} (as per **Figure 1B**) and SM*-CD is a [big P_{Cl}][small I_{Naleak}] system while CN*-CD is a [small P_{Cl}][big I_{Naleak}] system. CN-CD is non-minimal, but that negligibly affects its ATP-consumption (confirm by comparing CN-CD to MN-CD, **Table 2**). I_{Naleak} depends on P_{Na} and the electrodiffusive driving force (**Equations 2 and 3**). Though the Na^+ driving force is 21 mV bigger in SM*-CD than CN*-CD at their respective V_{rest} , this is outweighed by P_{Na} being 67X smaller in SM*-CD. Because $I_{\text{pump}} = -I_{\text{Naleak}}$, “instantaneous” ($t=0$) I_{Na} (**Figure 3A,B**) directly shows the crucial I_{Naleak} difference that makes steady-state ATP-consumption by SM(*)-CD $<1/5.3$ that of CN(*)-CD (see also **Figure 9Ai**).

Generally-speaking, electrically-leakier systems deteriorate faster. Based on total leak permeabilities (see **Figure 8A**) SM*-CD ($40.3 \mu\text{m}^3/\text{s}$) is electrically-leakier than CN*-CD ($24.5 \mu\text{m}^3/\text{s}$). Why, then, the markedly slower anoxic rundown (gradient dissipation, V_m decay, swelling) of SM*-CD? Because in P-L/D systems, rate-limiting permeabilities govern rundown, not global electrical-conductivity (**Figure 3** legend provides more specifics). CN*-CD, with P_{Cl} small relative to (its major permeability) P_{K} , represents “generic neuron” where, famously, the [small P_{Cl}] slows anoxic swelling (Rungta et al 2015). Therefore, the physiologically noteworthy take-home from this rundown comparison is: in [big P_{Cl}] SMFs, the extremely small P_{Na} confers substantially more safety from anoxic swelling than [small P_{Cl}] does in [big I_{Naleak}] neurons.

A priori, in P-L/D systems with unchanging permeabilities, a pump-strength sufficient to sustain steady-state gradients is also sufficient to restore systems after anoxic dissipation. **Figure 3** confirms this. SM*-CD, being less dissipated than CN*-CD at 105 minutes, recovers sooner, but whatever the rundown time, full recovery is assured (not shown).

Electrophysiological lifestyles and excitable cell anoxia

Neurons can respond sensitively to small incoming currents because their input impedance is relatively large and their V_{rest} lies close to firing threshold. SMFs, by contrast, keep V_{rest} hyperpolarized far below firing threshold and their smaller input impedance (from [big P_{Cl}]) prevents firing except in response to large excitatory postsynaptic currents (EPSCs) that, in situ, occur infrequently.

During anoxia (**Figure 4**) the behaviors of the P-L/D systems adapted for these distinctive “electrophysiological lifestyles” differ to an extreme degree; neurons perish rapidly and SMFs remain safe for prolonged periods. **Figure 4** follows CN-CD and SM-CD anoxic rundowns for 50 min (details in legend). Post-anoxic restoration is addressed elsewhere (for CN-CD, see Dijkstra et al (2016) and for SM-CD, see Morris et al submitted); suffice it to say that in both models post-anoxic recovery occurs only if I_{pump} is restored before ectopic firing ceases.

Instantaneous pump-off depolarization in CN-CD depolarizes V_m to firing threshold (**Figure 4A**). As ectopic APs fire for ~ 1 minute, Na^+ and K^+ channel opening precipitously dissipates the cation gradients. In CN-CD, a pathological voltage-activated Cl^- channel (Rungta et al 2015) hastens swelling. At at 50 minutes, CN-CD is 3X its initial volume (versus 2X for CN*-CD).

50 minutes into anoxia, the condition of SM-CD (**Figure 4B**) is equally disastrous. Having generated a half minute ectopic firing burst, it too has swollen 3X (versus only 1.2X for SM*-CD). The massive cation fluxes via Na^+ and K^+ plus concurrently a catastrophically large $[\text{Cl}^- + \text{H}_2\text{O}]$ influx. A different story is evident, however, when comparing systems, say, 20 minutes into anoxia. For 22.7 minutes, anoxic SM-CD avoids the Donnan-mediated swelling catastrophe, its prolonged rundown thanks to its hyperpolarized V_{rest} plus [small I_{Naleak}]. Ancillary to [small I_{Naleak}], moreover, its pump-off depolarization is small.

SMFs require [big P_{Cl}] as part of their no-added-cost “low-internal-resistance Donnan battery”; the feature keeping V_m near V_{rest} during (non-EPSC) current perturbations. Until firing threshold is reached, that [big P_{Cl}] is unproblematic. During those 22.7 minutes, in spite of [big P_{Cl}], the minimal P-L/D system keeps Cl^- influx small because Cl^- feels no driving force at $t=0$ (steady-state $E_{Cl}=V_{rest}$ and during rundown E_{Cl} tracks V_m). Though SM-CD’s P_K is small (compared to CN-CD), $P_K \gg P_{Na}$, so Na^+ entry through P_{Na} is mostly neutralized by K^+ exit. With Cl^-+H_2O entry minimal, SM-CD at 22.7 minutes into anoxia has swollen a mere 1.05X. The tightly coupled Cl^-+H_2O entry over this period is plotted at lower left, **Figure 3B**.

In SM-CD, anoxic depolarization from -75 mV $\rightarrow$ -65 mV takes 12 minutes. This accords well with the report (Clausen and Flatman 1977) that in ouabain-poisoned rat soleus fibers V_m runs down at ~ 10 mV/10 minutes.

In SM-CD, a ± 10 -fold ΔP_{Cl} does not affect V_{rest} and rundown at $10X P_{Cl}$ and $0.1X P_{Cl}$ takes 23.8 and 20.7 minutes to ectopic firing, respectively. Thus, its importance in stabilizing SMF V_{rest} notwithstanding, [big P_{Cl}] does not influence the value of V_{rest} (see **Figure 10**) and has little impact on its decay. As further evidence of the primacy, here, of [small I_{Naleak}]: a mere 27% P_{Na} decrease ($\rightarrow V_{rest} = -90$ mV), as illustrated in Morris et al submitted, lengthens rundown-to-ectopic firing from 22.7 to 34 minutes.

WD-CD: a counterfactual Pump-Leak dominated SM-CD analog

Many key physiological traits of SM-CD depend directly on [small I_{Naleak}] independent of [big P_{Cl}]: SM-CD’s low resting ATP-consumption and consequently its large pump-reserve (ratio of maximal to resting ATP-consumption), its strongly hyperpolarized V_{rest} , its slow anoxic rundown. It can be easy to lose sight of what [big P_{Cl}] contributes to Donnan dominated ([big P_{Cl}][small I_{Naleak}]) ion homeostasis.

To illustrate quantitatively, we use WD-CD (Weak Donnan, **Tables 1 and 2**), a counterfactual Pump-Leak dominated analog of SM-CD with the same input impedance and V_{rest} (-86 mV). Its assigned P_{Cl} is that of a physiologically-bonafide Pump-Leak dominated system (i.e., CN-CD) and its $P_{Na}:P_K$ ratio is that of SM-CD, but with larger absolute values to attain the input impedance of SM-CD. In counterfactual WD-CD, therefore, P_K (not P_{Cl}) is the major contributor to low input impedance. Like SM-CD, WD-CD is a minimal P-L/D system. Its [big I_{Naleak}] puts resting ATP-consumption at 3.5X that of SM-CD; its pump-reserve is only 3.1-fold. Though spending 3.5X more for steady-state Na^+ extrusion than SM-CD, its $[Na^+]_i$ is almost twice that of SM-CD, leaving it less able than SM-CD to support SMF’s E_{Na} -dissipating Na^+ -transporters.

In [big P_{Cl}] SM-CD, excitability modulation is straightforward (ΔP_{Cl} leaves ATP-consumption and V_{rest} unchanged) but WD-CD modulation is complex. Altering its input impedance via $\Delta P_K \rightarrow \Delta V_{rest}$. Modulating it with no ΔV_{rest} requires changing P_K and P_{Na} at fixed ratio. Any $\uparrow P_{Na} \rightarrow \uparrow$ ATP-consumption.

Ischemic episodes occur in the normal course of events for SMFs, making SM-CD’s exceptionally slow anoxic rundown a “time-is-money” benefit. Slow rundown makes it likelier that blood-flow resumes before ectopic firing triggers damaging contractures and cytotoxic swelling. **Figure 5** shows WD-CD anoxic rundown. Thanks to [small P_{Cl}] and initially zero Cl^- driving force, swelling prior to ectopic-APs is inconsequential (a neuron-like attribute). Nevertheless, due to its [big I_{Naleak}] WD-CD rundown-to-firing is 4X faster than in SM-CD (-75 $\rightarrow$ -65 mV in 2.9 minutes versus 12 min for SM-CD). Exacerbating this perilous rapidity is WD-CD’s 5.5 mV pump-off depolarization (versus <2 mV in SM-CD).

Evolution chose Donnan dominated ion homeostasis for SMFs

Like a neuron, a “WD-CD SMF” must continually “pay the pump” a) for its costlier V_{rest} and b) for most (i.e., the non- P_{Cl} portion) of its resting conductance. Like a neuron, WD-CD, is vulnerable to ischemia. An organism using Pump-Leak dominated “WD-CD muscles” for all its mostly-quiescent SMFs would

chronically elevate its whole-body expenditure, jeopardizing fuel supplies for contractility. **Figure Sup01C** shows that WD-CD (see the ATP-consumption trace) handles the 1200 AP stress-test as well as SM-CD. Its base-line energy consumption, however, is >3.5X higher.

For neurons and the whole-organism that supplies their ATP, payback for use of a costly Pump-Leak dominated system is their agile, nuanced integration of diverse information-rich electrical inputs. This merits the expenditure and the added vulnerability. By leaving SMF decision-making (when to contract?) entirely to remotely-located groupings of high-cost neurons, vertebrate SMFs could benefit from their robust low-cost Donnan dominated homeostasis strategy, allowing more ATP for contractility.

SMFs' [small I_{Naleak}] checks treacherous Donnan forces, while fostering the maintenance of a large pump-reserve and a strongly hyperpolarized, exceptionally low-cost V_{rest} that, thanks to SMF's "pre-paid" Cl^- -battery (i.e. $[\text{big } P_{\text{Cl}}] + [\text{Donnan effectors}]$), is not readily perturbed.

The slow ion homeostatic response to a fast SMF electrical event

The canonical ionic perturbation of SMF ion homeostasis is a cholinergic motorneuron-triggered AP (**Figure 2B**). For 3 minimal P-L/D models, Donnan dominated SM-CD (green), its ischemic (30% pump-strength) variant, LA-CD (blue), and (counterfactual) Pump-Leak dominated WD-CD (red) (see **Tables 1** and **2**), **Figure 6** details the response to an EPSC-triggered AP. Here, overlying the SM-CD AP of **Figure 2B** are almost-identical LA-CD and WD-CD APs. Time-axes and the various Y-axes are varied to resolve fast AP-related perturbations and the slower P-L/D responses. Single-AP ion and volume responses would be immeasurably small, but **Figure 2A,B** showed (for SM-CD) that the cumulative perturbation from 1200 APs elicits larger-magnitude P-L/D responses of nearly the same duration as for one AP. By 1200-APs in SM-CD (**Figure 2A**), $[\text{Na}^+]_i$ rises almost 25 mM above steady-state whereas post-one-AP, $[\text{Na}^+]_i$ increases <0.05 mM. For contraction-inhibited SMFs (current or voltage-clamped), a new 4-microelectrode technique enables simultaneous monitoring of multiple $[\text{ion}]_i$ (Heiny et al 2019; DiFranco et al 2019; for measurably-large perturbations, an AP train (not a single AP) would be needed).

Figure 6 dissects the underlying P-L/D processes.

The ATP-consumption trajectories are straightforward: after rapid double-jumps (responding to $\uparrow [\text{Na}^+]_i$ from the EPSC and AP), pump activity decays monotonically to steady-state over several minutes. WD-CD's trajectory is off-scale near 180 amol/s but otherwise like that of SM-CD (see Y-axis ranges, double-jump boxes) and so the Na^+ -extrusion and K^+ -uptake (difference) trajectories for SM-CD and WD-CD overlap, while weaker pump-strength LA-CD lags.

Return to steady-state $[\text{Cl}^-]$ and Vol_{cell} , rather than being monotonic, involves a single oscillation, reflecting the interplay of P-L/D systems' two sensor/effector processes. Once AP channels close, $V_m(t)$ reflects hyperpolarizing $I_{\text{pump}}(t)$. After 1200 APs in SM-CD (**Figure 2A**; see also **Figure Sup01B,Cii**), $V_m(t)$ hyperpolarizes and temporally aligned oscillations of $[\text{Cl}^-]_i(t)$ and $\text{Vol}_{\text{cell}}(t)$ occur as in **Figure 6**, but writ large.

All 3 models oscillate, their distinctive features revealing the forces at work (detailed in legend). Why the oscillation? Open AChR channels support mostly Na^+ entry, then Nav channels open before Kv channels. Immediately after any excess Na^+ entry (see AP-induced H_2O blip), cells are Na^+ and H_2O and Cl^- -overloaded. $[\text{Na}^+]_i$ -stimulated electrogenic I_{pump} extrudes 3Na^+ per 2K^+ imported, slightly hyperpolarizing the V_m (top panel, right). Shortly, the ongoing Na^+ -extrusion makes the swollen cells shrink below normal because the pump keeps expelling 3Na^+ per 2K^+ imported, and neutralizing- Cl^- plus osmo-balancing- H_2O accompany the extruded Na^+ (i.e. they exit the cell). $[\text{Cl}^-]_i$ and Vol_{cell} thus drop below their steady-state levels until, as $[\text{Na}^+]_i$ continues falling, I_{pump} and I_{Naleak} equalize and steady-state is re-attained.

The ion homeostatic feedback oscillation of $\text{Vol}_{\text{cell}}(t)$ and $[\text{Cl}^-]_i(t)$, we call a "Donnan bounce". The $\text{Vol}_{\text{cell}}(t)$ panel (right) shows that the Donnan bounce amplitude of Pump-leak dominated WD-CD is

$<1/10^{\text{th}}$ that of Donnan dominated SM-CD. A P-L/D system's Donnan bounce is bigger when P_{Cl} is bigger and slower when pump-strength is weaker.

Based on the **Figure 6** single-AP responses, one might ask:

1) Since WD-CD performs well post-AP, why would Pump-Leak dominated neurons (say) not go a step further, eliminate Cl^- channels, and use Pump-Leak homeostasis? The answer: this could work only if AP-related Na^+ and K^+ fluxes and the Na^+ and K^+ driving forces were precisely adjusted to yield zero excess Na^+ entry (AP-induced “ H_2O blips” would drop fully) AND if pumps were electroneutral (electrogenic pumping polarizes V_m , altering any perfectly-tuned driving forces). This zero- P_{Cl} system would have zero physiological latitude.

2) WD-CD experiences a smaller H_2O blip and thus a smaller Donnan bounce; is that not preferable? Inside a cranium, $\Delta\text{Vol}_{\text{cell}}$ is undesirable, but for SMFs the $\Delta\text{Vol}_{\text{cell}}$ of SM-CD is vanishingly small, physiologically-speaking, whereas WD-CD's chronic $3.5\times\uparrow\text{ATP}$ -consumption is an untenably large differential that, for low-duty-cycle SMFs, disqualifies the Pump-Leak dominated analog even ignoring its overly-fast anoxic rundown (**Figure 5B**).

SM-CD, ion homeostatic thresholds and bistability

SMF pump-strength varies up/down through hormonal modulation and misadventure (interrupted blood flow, toxins, sarcolemma bleb-damage) (Pirkmajer and Chibalin 2016). Given SMF's interacting non-linear Na^+ -flux mechanisms ($I_{\text{pump}}([\text{Na}^+]_i)$ and $I_{\text{Nav}}(V_m)$) (**Figure 9A,C**), and the non-linear driving forces acting on permeant ions, it is to be expected that fibers' P-L/D steady-states would vary non-linearly with pump-strength. Bifurcation plots for SM-CD (**Figure 7**) encapsulate these non-linearities.

For neurons in general and CN-CD in particular (Dijkstra et al 2016), it was shown (Hubel et al 2014) that pump-strength changes cause smoothly-varying (albeit non-linear) changes in steady-state values, until a system-specific unstable threshold is encountered. Here we examine this property for SM-CD. **Figure 7Aii** is the SM-CD destabilization trajectory (normal-pump-strength $\rightarrow$ saddle-node-pump-strength). Once spontaneous destabilization (ectopic firing) commences, the minimal-pump-strength system quickly degrades then slowly continues (not to DE, since pump-strength >0) to a “DE-like” pathological steady-state. SM-CD can now only return to a physiological steady-state (**Figure Sup02 Ci,ii,iii**) if it up-regulates its pump-strength ($=\uparrow\uparrow$ hyperpolarizing I_{maxpump}) enough to repolarize itself to a different unstable threshold ($\rightarrow$ more ectopic firing) from which (post-APs) it repolarizes to a physiological steady-state. This bistable two-thresholds scenario (“ion homeostatic excitability”) is a feature of excitable P-L/D systems (Hubel et al 2014; Morris 2018).

The parameter-specific bifurcation plots for a P-L/D system coincide; to know SM-CD's steady-state V_m , $[\text{Na}^+]_i$, Vol_{cell} , etc at, say, 10% pump-strength, consult each plot at that X-axis value. Furthermore, SM-CD/CN-CD comparisons are informative because their 100% pump-strength and C_m values are identical. For SM-CD, the lowest pump-strength able to sustain a physiological steady-state is marginally $<8\%$ (the saddle-node is $\sim 7.9\%$). Comparison of saddle-node pump-strengths ($\sim 8\%$ (SM-CD), $\sim 65\%$ (CN-CD)) re-emphasizes the impressive ion homeostatic robustness of SM-CD. Due to $3\times\uparrow\text{Nav}$ density, V_m at SM-CD's saddle-node is less depolarized than for CN-CD (**Figure 7Ai**); but note that SM-CD, at its saddle-node, though profoundly Na^+ -loaded, is hardly swollen (**7Bi,Cii**). Thus, unlike CN-CD, SM-CD can be profoundly ischemic and Na^+ -loaded with very little swelling. In humans, various methodologies including ^{23}Na -proton-MRI, point to “non-osmotic Na^+ -loading” in connection with myotonia (Weber et al 2006), over-exertion pain (Yu et al 2013), anaerobic exertion (Hammon et al 2015), and the chronically Na^+ -overloaded fibers of DMD patients (Gerhalter et al 2019).

The Vol_{cell} plot (**Figure 7Ci**) emphasizes the far-from-equilibrium state of healthy cells and the potency of the Donnan forces held in check by ion homeostasis by plotting the full range between DE and

physiological Vol_{cell} (black line) levels. **7Cii,iii** then bring the focus to the physiological region. Very little swelling is tolerated by CN-CD, but even SM-CD, whose saddle-node is reached at $\sim 2088 \mu\text{m}^3$, swells only $\sim 5\%$ ($\uparrow \text{Vol}_{\text{cell}}$). Working SMFs can safely withstand greater substantial swelling at healthier pump-strengths with Na^+ -transporters operative (Usher-smith et al 2009; Lindinger et al 2011).

The **Figure 7Aii** trajectory follows SM-CD to a degraded ATP-consuming pathological steady-state. If (implausibly) a SMF survived the ectopic firing/contractures and stabilized there, it would occupy a pathological steady-state point on the continuum (blue) directly above the black X. Recovery would require this degraded system to up-regulate its pump-strength to #. For completeness, **Figure Sup02Cii,iii** shows SM-CD's $V_m(t)$ trajectory when pump-strength is increased from 338% to 339%(#), whence it destabilizes and converges on the physiological steady-state continuum (directly below #).

Though peril clearly awaits if a saddle-node is encountered, ischemic SMFs are profoundly safer than ischemic neurons. If CN-CD drops 100% $\rightarrow$ 65%, only doubling its pump-strength (pink #) will save it. By contrast, SM-CD's physiological continuum extends down to 8%; over this entire ischemic range, any pump-strength improvement, no matter how small, moves the system smoothly back towards 100% (**Figure 7Bii**). SMFs commonly experience transient benign $\downarrow$ blood-flow (e.g., from sitting on one's foot). The slowness of anoxic/ischemic rundown times (**7Aii** inset), together with **7Bii** (recoverability) signifies that resting SMFs are well adapted to prolonged deep ischemic bouts (provided bouts end before myotonic firing commences and/or before motorneurons trigger APs).

Bifurcation analysis of SM-CD is congruent with the clinical situation, compartment syndrome, where, a normally-robust system harbours a treacherous threshold. Compartment syndrome describes progressive potentially life-threatening (gangrene) muscle ischemia, usually involving a limb, typically post-trauma. Johnstone and Ball (2019) indicate that "membrane injury and subsequent significant dysfunction is the central factor leading to irreversible {SMF} injury" and emphasize that because SMFs' remarkable tolerance of progressive ischemia ends abruptly, a fundamental requirement for better diagnoses is a means (intramuscular pH, perhaps) of identifying the ischemic threshold.

SM-CD dynamic range: up-regulated pumps and/or down-regulated P_{Na}

Intermittently, locomotory, thoracic, vocal, diaphragm and other muscles experience upsurges of ion homeostatic demand. **Figure 2A** showed a realistic hyper-stimulation of V-gated channels. *In situ*, Na^+ -transporters too would need to be supported. As Fraser and Huang (2004) show, use of NKCC, a cation-chloride cotransporter, would $\uparrow [\text{Na}^+]_i$, $\uparrow [\text{Cl}^-]_i$, $\uparrow \text{Vol}_{\text{cell}}$, $\downarrow [\text{K}^+]_i$, depolarize V_m and $\uparrow I_{\text{pump}}$. To extend its physiological dynamic range for excitability and transport, SM-CD shows us, a SMF could optimize its ATP consumption as described next.

Embedded in the physiological steady-state $[\text{Na}^+]_i$ continuum, **Figure 7Bi**, is an easily-overlooked P-L/D feature germane to physiological dynamic range. That plot's "boring" flattish region implies that no metabolic penalty is incurred for keeping pump-density chronically up-regulated (i.e., extra-large pump-reserves). Large $\uparrow$ pump-strength has little affect on $[\text{Na}^+]_i$ (**Figure 7Bii**). Increasing SM-CD pump-strength 100 $\rightarrow$ 200% (pump-reserve=11 $\rightarrow$ 22-fold, a level reported for rat soleus (Clausen 2015)) decreases $[\text{Na}^+]_i$ to 2.9 mM (**7Bii**), hyperpolarizes V_{rest} minimally (**7Ai**) leaving ATP-consumption at 51.1 amol/s (**Figure 9Bii**; X=2.9 mM, 200%-curve Y-axis intersect). **Figure Sup01A** illustrates that pump-reserve=22-fold accelerates post-stress-test recovery.

Temperate-climate amphibians and reptiles confront "scheduled" (i.e., seasonal) bouts of extreme ischemia. This requires modulation of excitable cell ion homeostasis (Pérez-Pinzón et al 1992; Donohue et al 2000). A testable prediction emerging from SM-CD is that, prior to entering hibernation, hibernators would increase SMF pump-density. Following the deep super-prolonged ischemic rundown of hibernation, this would expedite springtime recovery. If borne out, regulatory signaling systems could be sought. Another prediction: hibernators would simultaneously reduce P_{Na} for reasons outlined next.

A $\downarrow P_{Na}$ would improve a SMF's dynamic range. Why? Because $\downarrow P_{Na} \rightarrow \downarrow I_{pump}$. It also counteracts Na^+ -cotransporter-induced depolarization. For example, reducing P_{Na} from $0.3 \mu m^3/s$ to $0.22 \mu m^3/s$ hyperpolarizes SM-CD V_{rest} by 4 mV (to -90 mV; see **Figure 10**). Because $\downarrow [small I_{Naleak}] \rightarrow \downarrow I_{pump}$, ATP-consumption drops ~25% ($51.1 \text{ amol/s} \rightarrow 38.7 \text{ amol/s}$). This hypothetical change illustrates, yet again, the energetically pivotal role of SMFs' $[small I_{Naleak}]$ and hence of their small P_{Na} . Had we picked -90 mV as V_{rest} for SM-CD, the SM-CD/CN-CD ATP-consumption comparison would have been 1/7.0 instead of 1/5.3.

SMFs' small P_{Na} in GHK versus P-L/D settings

The immense physiological importance of SMFs' small P_{Na} has gone largely unrecognized. It is understood that V_{rest} depends on $P_{Na}:P_K$. It is understood that P_{Na} is the conduit for I_{Naleak} , the determinant of $I_{pump}(=-I_{Naleak})$. It is understood that P_{Na} thereby establishes the pump's small direct contribution (several millivolts) to V_{rest} (e.g., Sperelakis 2012). However, in spite of Fraser and Huang (2004)'s thorough exposition, it is under-recognized that for cells performing ion homeostasis (living cells) the zero-current Goldman-Hodgkin-Katz (GHK) equation is an inappropriate V_{rest} descriptor. This is not biophysical nit-picking.

Decreasing a small P_{Na} in a $P_K > P_{Na}$ GHK setting simply renders it increasingly inconsequential, and P_K more dominant. By contrast, in a P-L/D setting, as just illustrated for SM-CD, decreasing an already-small P_{Na} has physiologically consequential ramifications: $\downarrow P_{Na} \rightarrow \downarrow \text{ATP-consumption}$ and $\uparrow \text{pump-reserve}$. And in a transiently anoxic P-L/D system, $\downarrow P_{Na} \rightarrow \text{slower rundown and then faster recovery}$.

The unidentified P_{Na} of SMFs

In SMFs, P_{Na} (and ΔP_{Na}) has impressive physiological heft, but what is P_{Na} ? Neurons, smooth muscle and pancreatic cells use Na-Leak-Channel-Nonselective (NALCN) as a hormonally-regulated P_{Na} (Lu et al 2007; Senatore and Spafford 2013; Cochet-Bissuel et al 2014; Lutas et al 2018; Philippart et al 2018), but to date, NALCN has not been found in SMFs. Identifying the SMF P_{Na} and understanding its variability is considered critical in connection with myotonia (Metzger 2020). Persistent Nav channels (Gage et al 1989) might contribute, but since tetrodotoxin does not hyperpolarize V_{rest} in healthy fibers (e.g., Pickar et al 1991) this seems unlikely. The SMF P_{Na} could, like NALCN, be a cation channel (see Morris et al submitted). SMFs have several identified cation channels (see Metzger et al 2020) plus their routinely-detected unidentified mechanosensitive cation channel (Guharay and Sachs 1984; 1985; not a Piezo-channel (Suchyna 2017)). A precedent is worth recalling here: *Aplysia* neurons' serotonin and arachidonic acid regulated resting K^+ -channels (Belardetti et al 1986) are adventitiously mechanosensitive (e.g., see Vandorpe et al 1994; Morris and Horn 1991; Methfessel et al 1986; Zhang and Hamill, 2000; Zhang et al 2000; Morris 2012). Likewise for SMF mechanosensitive cation channels, but decades on, they have no attributed function (Lansman 2015; Suchyna 2017). A hypothesis worth pursuing: this cation channel does for SMFs what NALCN does for smooth muscle (e.g. Reinl et al 2018). Jointly, P_K and P_{Na} establish V_{rest} . Another critical and unresolved issue for SMF ion homeostasis, therefore, is whether/how they are jointly modulated (Kuba and Nohmi 1987; Donohue et al 2000; DiFranco et al 2015). Molecular identification of P_{Na} in SMFs could empower broad-reaching lines of biological and biomedical inquiry.

DISCUSSION

Ion homeostasis needs Donnan effectors

Comparative modeling of simple yet reassuringly realistic P-L/D systems here shows that, to serve distinctive electrophysiological lifestyles, SMFs and neurons evolved distinctive ion homeostatic strategies. Neuronal ion homeostasis biophysics was recently explained (Dijkstra et al 2016), but notwithstanding the foundational Fraser and Huang (2004) paper, SMF ion homeostasis mostly gets by-

passed. For animal cells in general, moreover, the physiological necessity of [Donnan effectors + P_{Cl}] for achieving ion homeostasis remains under-appreciated (Dmitriev et al 2019; Kay 2018).

To clarify the integral role of Donnan effectors in ion homeostasis, we outline first principles -- set point, sensor/effector feedbacks (**Figure 1A**)-- in their evolutionary context. This reveals ion homeostasis as a Pump-Leak/Donnan process. We depict “minimal” P-L/D systems at steady-state (**Figure 1B**), characterizing those P-L/D systems permeable mostly to anions as Donnan dominated, and those permeable mostly to cations as Pump-Leak dominated.

Computationally meshing the Fraser and Huang (2004) SMF model and the more accessible (compare pump descriptors) model of cortical neurons (Dijkstra et al 2017) we generated SM-CD, a minimal Donnan dominated excitable model for a SMF “ion homeostatic unit” (**Figure 8A**; **Tables 1, 2**).

Using SM-CD (and variant models) we demonstrate how low duty-cycle, low-excitability syncytial SMFs and more broadly, skeletal muscle, benefits from SMFs’ Donnan dominated strategy. The robust [big P_{Cl}][small I_{NaLeak}] process makes an ion homeostatic/electrophysiologic virtue of the unavoidable “vice” of Donnan effectors. Neurons’ Pump-Leak dominated [small P_{Cl}][big I_{NaLeak}] strategy, though far costlier and more imperiled by ischemia, is necessary for an information-processing lifestyle. For early vertebrates, the whole-organism contexts (**Table 2**) for these two strategies would have been an extreme version of the situation for humans: a low-cost Donnan dominated strategy for the body’s largest tissue and a costly pump-Leak dominated strategy for the small fractional mass of neurons. **Table 3** identifies and summarizes quantitatively several of the differences.

Ion homeostatic efficiency and SMF morphology

Even beyond **Table 3’s** list of CN-CD/SM-CD comparisons, ancestral vertebrates would have benefitted enormously from Donnan dominated ion homeostasis. This assertion assumes that then, as now, SMFs were syncytial (mammal SMFs have hundreds to thousands of nuclei (Ganassi et al 2018; for myonuclear domains, see Ross et al 2018). SM-CD and CN-CD have the same 2000 μm^2 surface area (SA) (C_m) and volume (**Figure 8A,Bii,Cii**). In situ however, SM-CD would not be mononucleate. It would be one of many cross-sectional slices of a “cylindrical” SMF comprised of hundreds to thousands of such slices (**Figure 8C,D**). By spherical geometry, 2000 μm^2 can fully enclose at most 8419 μm^3 , while by cylindrical geometry, the maximal volume encircled by 2000 μm^2 varies inversely with “ring”-width; for example, disposed as a 10 μm slice, 2000 μm^2 of sarcolemma (no slack) encircles ~32,000 μm^3 (see **Figure 8Di,ii**).

Syncytial morphology thus increases the cytoplasmic volume served (ion homeostatically) by a “unit” of sarcolemmal SA. SMFs are usually at steady-state, and crucially, steady-state ATP-consumption is independent of SA/Vol_{cell}. SA/Vol_{cell} does, however, matter during ionic perturbations: scaling linearly, for fixed-SA, both anoxic rundown times and ion homeostatic recovery times lengthen with $\uparrow$ Vol_{cell}. For low duty-cycle SMFs, the advantages conferred by slower rundown would, overall, substantially outweigh any drawbacks of slower recovery.

The SM-CD “ion homeostatic unit” and myonuclear domain volume

For mouse, data from Harris et al (2005); Mantilla et al (2008); Egner et al (2016) indicate: SMF radius ~15-30 μm , myonuclear density ~50/mm, myonuclear domain volume 15,000-30,000 μm^3 . For mouse SMFs, SM-CD’s 2000 μm^2 “ion homeostatic unit” would encircle more myoplasmic volume than depicted in **Figure 8Bii**. If mouse SMF nuclear density is ~1 nucleus/20 μm (~50 nuclei/mm $\rightarrow$ ~0.5 nucleus/10 μm), a plausible “volume-corrected” SM-CD would be a 20 μm slice of 15 μm radius. If it had SA=1885 μm^2 plus a slack 115 μm^2 for passive tension-buffering (1.06-fold swelling) that would be 2000 μm^2 encircling 14,137 μm^3 . (Sarcolemma’s dynamic tension-buffering (Sinha et al 2011; Lo et al 2016; Morris 2018) is not modeled here). A SM-CD unit such as this would be serving ~1 myonuclear domain.

The 2000 μm^2 “ion homeostatic unit” of SM-CD was picked with no consideration of myonuclear domain volume, but in retrospect, it is reasonable that a roughly comparable unit of SA would serve (and

be serviced by) one nucleus in both neurons and SMFs. For neurons, one nucleus serves soma, dendrites and axon, except when neurons become “giants” and either massively amplify their DNA-content in one nucleus (Lasek and Dower 1971) or become multinucleate (Mackie 1990).

Thus for these radically different cytomorphologies, the SA value taken from neurons to facilitate inter-model ion-flux comparisons seems roughly appropriate to associate with one myonucleus, but this remains a speculation; myonuclear domain volumes are routinely reported (e.g. Schwartz et al 2016) for SMFs, but we found no data on sarcolemmal-SA per myonuclear domain.

Switching off excitability for SM-CD robustness

An elevated risk accompanies the energetic benefits of syncytial morphology: loss of thousands of nuclei from one sarcolemmal tear. SMFs mitigate this danger via impressively reliable repair mechanisms (Andrews et al 2014; Barthélmy et al 2018; Manoharan et al 2019). Almost certainly the large pump-reserve facilitated by SMFs’ Donnan dominated strategy would be critical for re-establishing ion gradients post-tear. Imperative too would be diminished excitability. Whereas inexcitable SM*-CD recovers fully after >250 minutes of anoxic-rundown, at 25 minutes, excitable SM-CD having fired ectopically has swollen catastrophically. In the contexts of both ischemia and sarcolemmal tearing (McElhanon and Bhattacharya 2018; Hotfiel 2018), entry of Nav1.4 channels into slow-inactivated states (Webb et al 2009) needs to be studied.

P-L/D steady-states for real cells

“Set point” for ion homeostatic systems is a particular collection of steady-state values $\{V_{\text{cell}}, V_m, [\text{ion}]_i\}$ (Figure 1A). “Set point” may sound like a system “goal”, but we reiterate, deviations from “set point” values are not what is sensed operationally in a perturbed P-L/D system; the $\{V_{\text{cell}}, V_m, [\text{ion}]_i\}$ value-set is the CONSEQUENCE, not “the goal” of the P-L/D system’s feedback processes.

Clarity here makes it easier to see how, in large real cells (unlike in models) non-negligible distances and real diffusion times will yield “fuzzy” steady-state value-sets without abrogating the P-L/D concepts. Consider a quiescent 5 cm sartorius SMF or a 50 cm motoneuron. There is ad libitum ATP, axial resistance, and the densities of leak-channels and pumps are inhomogeneous. All along the cell, the P-L/D systems’ two sensor/effector mechanisms (Figure 1A) are continually sensing/ffecting. According to local densities of $P_{\text{Cl}}, P_{\text{Na}}, P_{\text{K}}$, pump, $[\text{Na}^+]_i$, sensing by the pumps affects I_{pump} and simultaneously, deviations from osmo-balance and compartment neutrality are stochastically/electrostatically sensed/countered via passive H_2O and Cl^- fluxes. At, say, the axon terminal, “ V_{rest} ” will likely differ from “ V_{rest} ” 50 cm away at the soma. Thus, at steady-state there would be standing $[\text{ion}]_i$ gradients and steady-state axial currents. In quiescent SMFs, with their endplate specializations and extensive t-tubular invaginations contiguous with the sarcolemma (Pedersen et al 2001; Fraser et al 2011), true steady-state will likewise be spatially inhomogeneous. Rhythmically-active excitable cells’ ion homeostatic steady-states are limit cycles (see Cha and Noma, 2012); thus, P-L/D set points can also be inhomogeneous in the temporal domain.

Excitable cells and P-L/D biotechnology

Symmorphosis (Fogarty and Sieck 2019), the evolutionary concept of economy of biological design, predicts that structural properties will be matched to functional demands. To analyze excitable cell ion homeostasis via a comparable conceptual filter, an ideal case would be the electric fish *Electrophorus electricus* (Gallant et al 2014; Catania 2015, 2019), given its low-duty-cycle syncytial SMFs and (derived evolutionarily from them) 3 subclasses of high-duty-cycle syncytial electrocytes (Traeger et al 2017). Available molecular (e.g., Nav channels, Kv, pumps) and morphological data for both cell types (Ching et al 2015, 2016; Thornhill et al 2003; Schwartz et al 1975; Machado et al 1983) could be

augmented with comparative proteomics (e.g., how much if any CIC-1 is expressed in electrocytes?). Molecular evolution data are available for O₂-delivery (globin-dependent) to SMFs and the electrocyte sub-classes (Tian et al 2017). Questions of ion homeostatic design economy/robustness/agility are intriguing from the perspective of evolutionary biology, and also increasingly, in the context of creative new biomimetic ventures such as the bioinspired engineering of “soft power sources” (Schroeder et al 2017) and the development of optogenetic skeletal muscle bioactuators and resilient self-healing muscle-machine interfaces (Raman et al 2016, 2017, 2019).

Prospects

Recently-developed cell-physiological techniques (Heiny et al 2019; DiFranco et al 2019) could make it possible to obtain concurrent SMF [ion]_i data (plus V_m(t)) along the lines of **Figure 2A**, while for whole tissue in humans, MRI advances are allowing for the non-invasive probing of ion homeostatic parameters in healthy, injured and diseased subjects (e.g., Hammon et al 2015; Dahlmann et al 2016, Gerhalter et al 2017, 2019; Zhang et al 2020). The accessible theoretical framework process presented here for SMF ion homeostasis as a P-L/D process should help guide investigative directions and facilitate interpretation of new data streams.

Though SM-CD is radically simple, adding and modifying components as appropriate is entirely feasible. A little-exploited but thoroughly rigorous cardiomyocyte CD-approach model (Cha and Noma 2012) demonstrates that even with a plethora of channels, transporters, compartments and binding reactions, a properly-designed CD-approach model will converge on steady-state. But, even in its present minimal state, SM-CD has provided comparative physiological insight relevant to vertebrate evolution. It also helps explain how the ischemic membrane-damaged SMFs of DMD patients can survive for decades (Morris et al submitted). Given appropriate refinements, SM-CD modeling should be helpful in connection with muscle fatigue, endurance, trauma, volume regulation, ischemia-reperfusion injury, ion channelopathies, hibernation, cold tolerance, sarcopenia and more (e.g., Donohue et al 2000; Lindinger et al 2011; dePaoli et al 2013; Yu et al 2013; Clausen 2015; Ammar et al 2015; Bækgaard Nielsen et al 2017; Boërio et al 2018; Hostrup and Bangsbo 2017; Hotfiel et al 2018; Cannon 2018; Copithorne and Rice 2019; Li et al 2020; Metzger et al 2020; Altamura et al 2020; Surkar et al 2020; Thoma et al 2020).

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 (**Figure 8, 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; see **Figure Meth 01C,D**) would likely have a smaller SA/V (surface area to volume) ratio than SM-CD (thus, slower Δ[ion]_i dynamics). The SM-CD membrane encloses a fixed quantity of Donnan effectors: A⁻, impermeant monovalent anions. The extracellular medium has a small 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 μm³ and a constant C_m corresponding to a SA of 2000 μm². As such, resting state cells are flaccid, i.e., not maximally inflated (**Figure 8**). Lipid bilayers tolerate little lateral expansion, but unless a model-cell inflated to spherical its membrane would not be subject to tension and rupture. 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 9**). 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_m** 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 4** 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 (**Figure 9**) 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 H₂O 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 et al 2004; 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

$$passive Na^+ influx + active Na^+ outpump = zero$$

AND

$$passive K^+ efflux + active K^+ inpump = zero.$$

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 (**Figure 10**). $V_{rest} = -86$ 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-CM 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 $[intracellular\ anions] - [intracellular\ cations]$:

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

$\Delta c = 0.00886$ 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) (see **Figure 1A top**). 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:

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

Computational methods

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

ACKNOWLEDGEMENTS

We acknowledge financial support from Natural Sciences and Engineering Council (Canada) (BJ and JJW) and support from the Ottawa Health Research Institute (CEM).

COMPETING INTERESTS

There are no competing interests.

REFERENCES

- Allen DG, Whitehead NP, Froehner SC. 2016. Absence of dystrophin disrupts skeletal muscle signaling: Roles of Ca²⁺, reactive oxygen species, and nitric oxide in the development of muscular dystrophy. *Physiol Rev.* 96:253-305. doi: 10.1152/physrev.00007.2015
- Altamura C, Desaphy JF, Conte D, De Luca A, Imbrici P. 2020. Skeletal muscle CIC-1 chloride channels in health and diseases. *Pflugers Arch: European Journal of Physiology* 472:961–975. doi: 10.1007/s00424-020-02376-3
- Ammar T, Lin W, Higgins A, Hayward LJ, Renaud JM. 2015. Understanding the physiology of the asymptomatic diaphragm of the M1592V hyperkalemic periodic paralysis mouse. *J Gen Physiol.* 146:509-525. doi: 10.1085/jgp.201511476
- Andrews NW, Almeida PE, Corrotte M. 2014. Damage control: cellular mechanisms of plasma membrane repair. *Trends Cell Biol.* 24:734-742. doi:10.1016/j.tcb.2014.07.008
- Bækgaard Nielsen O, de Paoli FV, Riisager A, Pedersen TH. 2017. Chloride channels take center stage in acute regulation of excitability in skeletal muscle: implications for fatigue. *Physiology (Bethesda).* 32:425-434. doi: 10.1152/physiol.00006.2015
- Barthélémy F, Defour A, Lévy N, Krahn M, Bartoli M. 2018. Muscle cells fix breaches by orchestrating a membrane repair ballet. *J Neuromuscul Dis.* 5:21-28. doi: 10.3233/JND-170251
- Belardetti F, Schacher S, Kandel ER, Siegelbaum SA. 1986. The growth cones of *Aplysia* sensory neurons: Modulation by serotonin of action potential duration and single potassium channel currents. *Proc Natl Acad Sci U S A.* 83:7094-7098. doi: 10.1073/pnas.83.18.7094
- Bennett MVL. Electric Organs. 1971. In: Hoar WS, Randall DJ, editors. *Fish Physiology*. 5. New York: Academic Press; p. 347-491. doi: 10.1016/S1546-5098(08)60051-5
- Boério D, Corrêa TD, Jakob SM, Ackermann KA, Bostock H, Z'Graggen WJ. 2018. Muscle membrane properties in A pig sepsis model: Effect of norepinephrine. *Muscle Nerve.* 57:808-813. doi: 10.1002/mus.26013
- Boucher P-A, Joós B, Morris CE. 2012. Coupled left-shift of Nav channels: modeling the Na⁺ loading and dysfunctional excitability of damaged neurons. *J. Comput. Neurosci.* 33:301-319. doi: 10.1007/s10827-012-0387-7
- Casane D, Laurenti P. 2013. Why coelacanths are not 'living fossils': a review of molecular and morphological data. *Bioessays* 35:332-338. doi: 10.1002/bies.201200145
- Cannon SC. 2018. Sodium channelopathies of skeletal muscle. *Handb Exp Pharmacol.* 246:309-330. doi: 10.1007/978-3-319-90284-5
- Catania KC. 2015. Electric eels use high-voltage to track fast-moving prey. *Nat Commun.* 6:8638. doi: 10.1038/ncomms9638
- Catania KC. The astonishing behavior of electric Eels. 2019. *Front Integr Neurosci.* 13:23. doi: 10.3389/fnint.2019.00023
- Cha CY, Noma A. 2012. Steady-state solutions of cell volume in a cardiac myocyte model elaborated for membrane excitation, ion homeostasis and Ca²⁺ dynamics. *J Theor Biol.* 307:70-81. doi: 10.1016/j.jtbi.2012.04.025

- Ching B, Woo JM, Hiong KC, Boo MV, Choo CY, Wong WP, Chew SF, Ip YK. 2015; Na⁺/K⁺-ATPase α -subunit (nka α) isoforms and their mRNA expression levels, overall Nka α protein abundance, and kinetic properties of Nka in the skeletal muscle and three electric organs of the electric eel, *Electrophorus electricus*. PLoS One 10:e0118352. doi: 10.1371/journal.pone.0118352
- Ching B, Woo JM, Hiong KC, Boo MV, Wong WP, Chew SF, Ip YK. 2016. Voltage-Gated Na⁺ channel isoforms and their mRNA expression levels and protein abundance in three electric organs and the skeletal muscle of the electric eel *Electrophorus electricus*. PLoS One 11:e0167589. doi: 10.1371/journal.pone.0167589
- Clausen T. 2013. Quantification of Na⁺,K⁺ pumps and their transport rate in skeletal muscle: functional significance. J Gen Physiol. 142:327-345. doi:10.1085/jgp.201310980
- Clausen T. 2015. Excitation of skeletal muscle is a self-limiting process, due to run-down of Na⁺, K⁺ gradients, recoverable by stimulation of the Na⁺, K⁺ pumps. Physiol. Rep. 3. e12373. doi: 10.14814/phy2.12373
- Clausen T, Flatman JA. 1977. The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle. J Physiol. 270:383-414. doi: 10.1113/jphysiol.1977.sp011958
- Cochet-Bissuel M, Lory P, Monteil A. 2014. The sodium leak channel, NALCN, in health and disease. Front Cell Neurosci. 8: 132. doi: 10.3389/fncel.2014.00132
- Copithorne DB, Rice CL .2019.The effect of blood flow occlusion during acute low-intensity isometric elbow flexion exercise. Eur J Appl Physiol. 119:587-595. doi: 10.1007/s00421-019-04088-8
- Dahlmann A, Kopp C, Linz P, Cavallaro A, Seuss H, Eckardt K, Luft FC., Titze J, Uder M, Hammon M. 2016. Quantitative assessment of muscle injury by ²³Na magnetic resonance imaging. Springerplus. 5: 661. doi: 10.1186/s40064-016-2193-6
- de Paoli FV, Broch-Lips M, Pedersen TH, Nielsen OB. 2013. Relationship between membrane Cl⁻ conductance and contractile endurance in isolated rat muscles. J Physiol. 591:531-545. doi: 10.1113/jphysiol.2012.243246
- DiFranco M, Yu C, Quiñonez M, Vergara JL. 2015. Inward rectifier potassium currents in mammalian skeletal muscle fibres. J Physiol. 593:1213-1238. doi:10.1113/jphysiol.2014.283648
- DiFranco M, Quinonez M, Dziedzic RM, Spokorny AM, Cannon SC. 2019. A highly-selective chloride microelectrode based on a mercuracarborand anion carrier. Sci Rep. 9:18860. doi: 10.1038/s41598-019-54885-6
- Dijkstra K, Hofmeijer J, van Gils SA, van Putten MJ. 2016. A biophysical model for cytotoxic cell swelling. J Neurosci. 36:11881. doi: 10.1523/JNEUROSCI.1934-16.2016
- Dmitriev AV, Dmitriev AA, Linsenmeier RA. 2019. The logic of ionic homeostasis: Cations are for voltage, but not for volume. PLoS Comput Biol. 15:e1006894. doi: 10.1371/journal.pcbi.1006894
- Donohoe PH, West TG, Boutilier RG. 2000. Factors affecting membrane permeability and ionic homeostasis in the cold-submerged frog. J Exp Biol. 203(Pt 2):405-414. ISSN: 1477-9145.
- Dutel H, Galland M, Tafforeau P, Long JA, Fagan MJ, Janvier P, Herrel A, Santin MD, Clément G, Herbin M. 2019. Neurocranial development of the coelacanth and the evolution of the sarcopterygian head. Nature 569:556-559. doi: 10.1038/s41586-019-1117-3
- Egner IM, Bruusgaard JC, Gundersen K. 2016. Satellite cell depletion prevents fiber hypertrophy in skeletal muscle. Development 143:2898-2906. doi: 10.1242/dev.134411

- Fogarty MJ, Sieck GC. 2019. Evolution and functional differentiation of the diaphragm muscle of mammals. *Compr Physiol*. 9:715-766. doi: 10.1002/cphy.c180012
- Fraser JA, Huang CL. 2004. A quantitative analysis of cell volume and resting potential determination and regulation in excitable cells. *J Physiol*. 559:459. doi: 10.1113/jphysiol.2004.065706
- Fraser JA, Huang CL. 2007. Quantitative techniques for steady-state calculation and dynamic integrated modelling of membrane potential and intracellular ion concentrations. *Prog Biophys Mol Biol*. 94:336-372. doi: 10.1016/j.pbiomolbio.2006.10.001
- Fraser JA, Huang CL, Pedersen TH. 2011. Relationships between resting conductances, excitability, and t-system ionic homeostasis in skeletal muscle. *J Gen Physiol*. 138:95-116. doi: 10.1085/jgp.201110617
- Fu Y, Struyk A, Markin V, Cannon S. 2011. Gating behaviour of sodium currents in adult mouse muscle recorded with an improved two-electrode voltage clamp. *J Physiol*. 589:525-546. doi: 10.1113/jphysiol.2010.199430
- Gage PW, Lamb GD, Wakefield BT. 1989. Transient and persistent sodium currents in normal and denervated mammalian skeletal muscle. *J Physiol*. 418:427-439. doi:10.1113/jphysiol.1989.sp017850Gage
- Gallant JR, Traeger LL, Volkeneing JD, Moffett H, Chen PH, Novina CD, Phillips Jr.GN, Anand R, Wells GB, Pinch M, Güth R, Unguez GA, Albert JS, Zakon HH, Samanta MP, Sussman MR. 2014. Genomic basis for the convergent evolution of electric organs. *Science* 344:1522-1525. doi:10.1126/science.1254432
- Ganassi M, Badodi S, Ortuste Quiroga HP, Zammit PS, Hinitis Y, Hughes SM. 2018. Myogenin promotes myocyte fusion to balance fibre number and size. *Nat Commun*. 9:4232. doi: 10.1038/s41467-018-06583-6
- Gerhalter T, Carlier PG, Marty B. 2017. Acute changes in extracellular volume fraction in skeletal muscle monitored by ²³Na NMR spectroscopy. *Physiol. Rep*. 5: e13380. doi: 10.14814/phy2.13380
- Gerhalter T, Gast LV, Marty B, Martin J, Trollmann R, Schüssler S, Roemer F, Laun FB, Uder M, Schröder R, Carlier PG, Nagel AM. 2019. ²³Na MRI depicts early changes in ion homeostasis in skeletal muscle tissue of patients with Duchenne muscular dystrophy. *J Magn Reson Imaging*. 50:1103-1113. doi: 10.1002/jmri.26681
- Guharay F, Sachs F. 1984. Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J Physiol*. 352:685-701. doi: 10.1113/jphysiol.1984.sp015317
- Guharay F, Sachs F. 1985. Mechanotransducer ion channels in chick skeletal muscle: the effects of extracellular pH. *J Physiol*. 363:119-134. doi: 0.1113/jphysiol.1985.sp015699
- Hamada K, Matsuura H, Sanada M, Toyoda F, Omatsu-Kanbe M, Kashiwagi A, Yasuda H. 2003. Properties of the Na⁺/K⁺ pump current in small neurons from adult rat dorsal root ganglia. *Br J Pharmacol*. 138:1517-1527. doi: 10.1038/sj.bjp.0705170
- Hammon M, Grossmann S, Linz P, Kopp C, Dahlmann A, Janka R, Cavallaro A, Uder M, Titze J. 2015. 3 Tesla (²³Na) magnetic resonance imaging during aerobic and anaerobic exercise. *Acad Radiol*. 22:1181-1190. doi: 10.1016/j.acra.2015.06.005
- Harris AJ, Duxson MJ, Butler JE, Hodges PW, Taylor JL, Gandevia SC. 2005. Muscle fiber and motor unit behavior in the longest human skeletal muscle. *J Neurosci*. 25:8528-8533. doi: 10.1523/JNEUROSCI.0923-05.2005
- Heiny JA, Cannon SC, DiFranco M. 2019. A four-electrode method to study dynamics of ion activity and transport in skeletal muscle fibers. *J Gen Physiol*. 151:1146-1155. doi: 10.1085/jgp.201912398

- Heiny JA, Kravtsova VV, Mandel F, Radzyukevich TL, Benziane B, Prokofiev AV, Pedersen SE, Chibalin AV, Krivoi II. 2010. The nicotinic acetylcholine receptor and the Na,K-ATPase alpha2 isoform interact to regulate membrane electrogenesis in skeletal muscle. *J Biol Chem.* 285:28614-28626. doi: 10.1074/jbc.M110.150961
- Helfman G, Collette B, Facey DE. 2009. *The Diversity of Fishes: Biology, Evolution and Ecology*, 2nd ed.. Blackwell Science, Malden, MA. pp. 48–49. ISBN: 978-1-405-12494-2
- Hille, B. 2001. *Ion Channels of Excitable Membranes*. 3rd edition Sinauer Associates, Sunderland, Massachusetts. ISBN: 978-0-87893-321
- Hotfiel T, Freiwald J, Hoppe MW, Lutter C, Forst R, Grim C, Bloch W, Hüttel M, Heiss R. 2018. Advances in delayed-onset muscle soreness (DOMS): Part I: Pathogenesis and diagnostics. *Sportverletz Sportschaden.* 32:243-250
- Hostrup M, Bangsbo J. 2017. Limitations in intense exercise performance of athletes - effect of speed endurance training on ion handling and fatigue development. *J Physiol.* 595:2897-2913. doi: 10.1113/JP273218
- Hotfiel T, Freiwald J, Hoppe MW, Lutter C, Forst R, Grim C, Bloch W, Hüttel M, Heiss R. 2018. Advances in delayed-onset muscle soreness (DOMS): Part I: Pathogenesis and diagnostics. *Sportverletz Sportschaden.* 32:243-250. doi: 10.1055/a-0753-1884
- Hübel N, Schöll E, Dahlem MA. 2014. Bistable dynamics underlying excitability of ion homeostasis in neuron models. *PLoS Comput Biol.* 10:e1003551. doi: 10.1371/journal.pcbi.1003551
- Janssen I, Heymsfield SB, Wang ZM, Ross R. 2000. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol* 89:81-88. doi: 10.1152/jappl.2000.89.1.81
- Jeng CJ, Fu SJ, You CY, Peng YJ, Hsiao CT, Chen TY, Tang CY. 2020. Defective gating and proteostasis of human ClC-1 chloride channel: molecular pathophysiology of myotonia congenita. *Front Neurol.* 11:76. doi: 10.3389/fneur.2020.00076
- Jentsch TJ, Pusch M. 2018. ClC chloride channels and transporters: structure, function, physiology, and disease. *Physiol Rev.* 98:1493-1590. doi: 10.1152/physrev.00047.2017
- Johnstone AJ, Ball D. 2019. Determining ischaemic thresholds through our understanding of cellular metabolism. In: Mauffrey C, Hak DJ, Martin III MP, editors. *Compartment Syndrome: A Guide to Diagnosis and Management (SpringerOpen)*, Chapter 4. doi: 10.1007/978-3-030-22331-1_4
- Johnston IA, Bower NI, Macqueen DJ. 2011. Growth and the regulation of myotomal muscle mass in teleost fish. *J Exp Biol.* 214:1617-1628
- Joos B, Barlow BM, Morris CE. 2018. Calculating the consequences of left-shifted Nav channel activity in sick excitable cells. *Handb Exp Pharmacol.*; 246:401-422 (Review). doi: 10.1007/164_2017_63
- Kager H, Wadman WJ, Somjen GG. 2000. Simulated seizures and spreading depression in a neuron model incorporating interstitial space and ion concentrations. *J Neurophysiol.* 84:495-512. doi: 10.1152/jn.2000.84.1.495
- Kay AR. 2017. How cells can control their size by pumping ions. *Front. Cell Dev. Biol.* 5:41. doi: 10.3389/fcell.2017.00041
- Kuba K, Nohmi M. 1987. Role of ion conductance changes and of the sodium-pump in adrenaline-induced hyperpolarization of rat diaphragm muscle fibres. *Br J Pharmacol.* 91:671-681. doi:10.1111/j.1476-5381.1987.tb11261.x

- Lansman JB. 2015. Utrophin suppresses low frequency oscillations and coupled gating of mechanosensitive ion channels in dystrophic skeletal muscle. *Channels (Austin)* 2015 9: 145–160. doi: 10.1080/19336950.2015.1040211
- Lasek RJ, Dower WJ. 1971. *Aplysia californica*: analysis of nuclear DNA in individual nuclei of giant neurons. *Science*. 172:278-280. doi: 10.1126/science.172.3980.278
- Lauf PK, Sharma N, Adragna NC. 2019. Kinetic studies of K-Cl cotransport in cultured rat vascular smooth muscle cells. *Am J Physiol Cell Physiol*. 316:C274-C284. doi: 10.1152/ajpcell.00002.2017
- Li RW, Deng Y, Pham HN, Weiss S, Chen M, Smith PN. 2020. Riluzole protects against skeletal muscle ischaemia-reperfusion injury in a porcine model. *Injury*. 51:178-184. doi: 10.1016/j.injury.2019.12.030
- Lindinger MI, Leung M, Trajcevski KE, Hawke TJ. 2011. Volume regulation in mammalian skeletal muscle: the role of sodium-potassium-chloride cotransporters during exposure to hypertonic solutions. *J Physiol*. 589:2887-2899. doi: 10.1113/jphysiol.2011.206730
- Lo HP, Hall TE, Parton RG. 2016. Mechanoprotection by skeletal muscle caveolae. *Bioarchitecture*. 6:22-27. doi: 10.1080/19490992.2015.1131891
- Lorenzo LE, Godin AG, Ferrini F, Bachand K, Plasencia-Fernandez I, Labrecque S, Girard AA, Boudreau D, Kianicka I, Gagnon M, Doyon N, Ribeiro-da-Silva A, De Koninck Y. 2020. Enhancing neuronal chloride extrusion rescues $\alpha 2/\alpha 3$ GABAA-mediated analgesia in neuropathic pain. *Nat. Commun.*11:869. doi: 10.1038/s41467-019-14154-6
- Lu B, Su Y, Das S, Liu J, Xia J, Ren D. 2007. The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. *Cell*. 129:371-383. doi: 10.1016/j.cell.2007.02.041
- Lutas A, Lahmann C, Soumillon M2, Yellen G. 2016. The leak channel NALCN controls tonic firing and glycolytic sensitivity of substantia nigra pars reticulata neurons. *Elife*. 5:e15271. doi: 10.7554/eLife.15271
- Machado RD, Miguens FC, Esquibel MA. 1983. Scanning electron microscopy of the electric organs of *Electrophorus electricus* L. I. Main organ. *Cell Tissue Res*. 234:641-653. doi: 10.1007/BF00218657
- Mackie GO. 1990. Giant axons and control of jetting in the squid *Loligo* and the jellyfish *Aglantha*. *Canadian Journal of Zoology* 68: 799-805. doi: 10.1139/z90-115
- Manoharan P, Song T, Radzyukevich TL, Sadayappan S, Lingrel JB, Heiny JA. 2019. KLF2 in myeloid lineage cells regulates the innate immune response during skeletal muscle injury and regeneration. *Science*. 17:334-346. doi: 10.1016/j.isci.2019.07.009
- McElhanon KE, Bhattacharya S. 2018. Altered membrane integrity in the progression of muscle diseases. *Life Sci*. 192:166-172. doi: 10.1016/j.lfs.2017.11.035
- Methfessel C, Witzemann V, Takahashi T, Mishina M, Numa S, Sakmann B. 1986. Patch clamp measurements on *Xenopus laevis* oocytes: currents through endogenous channels and implanted acetylcholine receptor and sodium channels. *Pflugers Arch*. 407:577-88. doi: 10.1007/BF00582635
- Metzger S, Dupont C, Voss AA, Rich MM. 2020. Central role of subthreshold currents in myotonia. *Ann Neurol*. 87:175-183. doi: 10.1002/ana.25646
- Miller C. 2015. In the beginning: a personal reminiscence on the origin and legacy of ClC-0, the 'Torpedo Cl' channel'. *J Physiol*. 593:4085-4090. doi: 10.1113/jphysiol.2014.286260

- Mink JW, Blumenschine RJ, Adams DB. 1981. Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am J Physiol.* 241:R203-R212. doi: 10.1152/ajpregu.1981.241.3.R203
- Morris CE, Horn R. 1991. Failure to elicit neuronal macroscopic mechanosensitive currents anticipated by single-channel studies. *Science.* 251:1246-1249. doi: 10.1126/science.1706535
- Morris CE. 2012. Why are so many channels mechanosensitive? In N. Sperelakis (Ed.) *Cell Physiology Source Book*, 4th Edn, pp 493-505. Elsevier. ISBN: 0-12-656977-0
- Morris CE, Boucher P-A, Joós B. 2012. Left-shifted Nav channels in trauma-damaged bilayer: primary targets for neuroprotective Nav antagonists? *Frontiers in Pharmacology* 3, 19. doi: 10.3389/fphar.2012.00019
- Morris CE. 2018. Cytotoxic swelling of sick excitable cells - impaired ion homeostasis and membrane tension homeostasis in muscle and neuron. *Curr Top Membr* 81:457-496. doi: 10.1016/bs.ctm.2018.06.001
- Morris CE, Wheeler JW, Joos B. Donnan dominated ion homeostasis and the longevity of ischemic Na⁺-loaded dystrophic skeletal muscle (*submitted*)
- Nielsen OB, Clausen T. 1997. Regulation of Na⁺-K⁺ pump activity in contracting rat muscle. *J Physiol.* 503:571-581. doi: 10.1111/j.1469-7793.1997.571bg.x
- Novak KR, Norman J, Mitchell JR, Pinter MJ, Rich MM. 2015. Sodium channel slow inactivation as a therapeutic target for myotonia congenita. *Ann Neurol.* 77:320-332. doi: 10.1002/ana.24331
- Pedersen TH, Macdonald WA, de Paoli FV, Gurung IS, Nielsen O. 2011. Comparison of regulated passive membrane conductance in action potential-firing fast- and slow-twitch muscle. *J Gen Physiol.* 134:323-337. doi: 10.1085/jgp.200910291
- Pedersen TH, Riisager A, de Paoli FV, Chen TY, Nielsen OB. 2016. Role of physiological ClC-1 Cl⁻ ion channel regulation for the excitability and function of working skeletal muscle. *J Gen Physiol.* 147:291-308. doi: 10.1085/jgp.201611582
- Pérez-Pinzón MA, Rosenthal M, Sick TJ, Lutz PL, Pablo J, Mash D. 1992. Downregulation of sodium channels during anoxia: a putative survival strategy of turtle brain. *Am J Physiol* 262:R712-R715. doi: 10.1152/ajpregu.1992.262.4.R712
- Philippart F, Khaliq ZM. 2018. G_{i/o} protein-coupled receptors in dopamine neurons inhibit the sodium leak channel NALCN *eLife* 7: e40984. doi: 10.7554/eLife.40984
- Pickar JG, Spier SJ, Snyder JR, Carlsen RC. 1991. Altered ionic permeability in skeletal muscle from horses with hyperkalemic periodic paralysis. *Am J Physiol.* 260:C926-C933. doi: 10.1152/ajpcell.1991.260.5.C926
- Pirkmajer S, Chibalin AV. 2016. Na,K-ATPase regulation in skeletal muscle. *Am J Physiol Endocrinol Metab.* 311:E1-E31. doi: 10.1152/ajpendo.00539.2015
- Rakowski RF, Gadsby DC, De Weer P. 1997. Voltage dependence of the Na/K pump. *J Membr Biol.* 155:105-112. doi:10.1007/s002329900162
- Rahmat S, Gilland E. 2014. Comparative anatomy of the carotid-basilar arterial trunk and hindbrain penetrating arteries in vertebrates. *The Open Anatomy Journal* 6:1-26. doi: 10.2174/1877609401406010001

- Raman R, Cvetkovic C, Uzel SG, Platt RJ, Sengupta P, Kamm RD, Bashir R. 2016. Optogenetic skeletal muscle-powered adaptive biological machines. *Proc Natl Acad Sci U S A*. 113:3497-3502. doi: 10.1073/pnas.1516139113
- Raman R, Grant L, Seo Y, Cvetkovic C, Gapinske M, Palasz A, Dabbous H, Kong H, Pinera PP, Bashir R. 2017. Damage, healing, and remodeling in optogenetic skeletal muscle bioactuators. *Adv Healthc Mater*. 6:e1700030. doi: 10.1002/adhm.201700030
- Raman R. 2019. Modeling muscle. *Science*. 363:1051. doi: 10.1126/science.aaw3611
- Reinl EL, Zhao P, Wu W, Ma X, Amazu C, Bok R, Hurt KJ, Wang Y, England SK. 2018. Na⁺-Leak Channel, Non-Selective (NALCN) regulates myometrial excitability and facilitates successful parturition. *Cell Physiol Biochem*. 48:503-515. doi: 10.1159/000491805
- Rolfe DF, Brown GC. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev*. 77:731-758. doi: 10.1152/physrev.1997.77.3.731
- Ross JA, Levy Y, Svensson K, Philp A, Schenk S, Ochala J. 2018. SIRT1 regulates nuclear number and domain size in skeletal muscle fibers. *J Cell Physiol*. 233:7157-7163. doi:10.1002/jcp.26542
- Ruff RL. 2011. Endplate contributions to the safety factor for neuromuscular transmission. *Muscle Nerve* 44:854-861. doi: 10.1002/mus.22177
- Rungta RL, Choi HB, Tyson JR, Malik A, Dissing-Olesen L, Lin PJC, Cain SM, Cullis PR, Snutch TP, MacVicar BA. 2015. The cellular mechanisms of neuronal swelling underlying cytotoxic edema. *Cell* 161:610. doi: 10.1016/j.cell.2015.03.029
- Schroeder TBH, Guha A, Lamoureux A, VanRenterghem G, Sept D, Shtein M, Yang J, Mayer M. 2017. An electric-eel-inspired soft power source from stacked hydrogels. *Nature* 552:214-218. doi: 10.1038/nature24670
- Schwartz IR, Pappas GD, Bennett MVL. 1975. The fine structure of electrocytes in weakly electric teleosts. *J Neurocytol*. 4:87-114. ISSN: 1573-7381, 0300-4864
- Schwartz LM, Brown C, McLaughlin K, Smith W, Bigelow C. 2016. The myonuclear domain is not maintained in skeletal muscle during either atrophy or programmed cell death. *Am J Physiol Cell Physiol*. 311:C607-C615. doi:10.1152/ajpcell.00176.2016
- Senatore A, Spafford JD. 2013. A uniquely adaptable pore is consistent with NALCN being an ion sensor. *Channels (Austin)*. 7:60-68. doi: 10.4161/chan.23981
- Sinha B, Köster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, Stan RV, Butler-Browne G, Védie B, Johannes L, Morone N, Parton RG, Raposo G, Sens P, Lamaze C, Nassoy P. 2011. Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell*. 144:402-413. doi:10.1016/j.cell.2010.12.031
- Sperelakis N (2012) Origin of resting membrane potentials. In N. Sperelakis (Ed.) *Cell Physiology Source Book*, 4th Edn, pp 121- 145. Elsevier. ISBN: 0-12-656977-0.
- Steinmeyer K, Ortland C, Jentsch TJ. 1991. Primary structure and functional expression of a developmentally regulated skeletal muscle chloride channel. *Nature*. 354:301-304. doi: 10.1038/354301a0.
- Suchyna TM. 2017. Piezo channels and GsMTx4: Two milestones in our understanding of excitatory mechanosensitive channels and their role in pathology. *Prog Biophys Mol Biol*. 130:244-253. doi:10.1016/j.pbiomolbio.2017.07.011

- Surkar SM, Bland MD, Mattlage AE, Chen L, Gidday JM, Lee JM, Hershey T, Lang CE. 2020. Effects of remote limb ischemic conditioning on muscle strength in healthy young adults: A randomized controlled trial. *PLoS One*. 15: e0227263. doi: 10.1371/journal.pone.0227263
- Thoma A, Akter-Miah T, Reade RL, Lightfoot AP. 2020. Targeting reactive oxygen species (ROS) to combat the age-related loss of muscle mass and function. *Biogerontology* 21:475-484. doi: 10.1007/s10522-020-09883-x
- Tian R, Losilla M, Lu Y, Yang G, Zakon H. 2017. Molecular evolution of globin genes in Gymnotiform electric fishes: relation to hypoxia tolerance. *BMC Evol Biol*. 17:51. doi: 10.1186/s12862-017-0893-3
- Traeger LL, Sabat G, Barrett-Wilt GA, Wells GB, Sussman MR. 2017. A tail of two voltages: Proteomic comparison of the three electric organs of the electric eel. *Sci Adv*. 3: e1700523. doi: 10.1126/sciadv.1700523
- Usher-Smith JA, Huang CL, Fraser JA. 2009. Control of cell volume in skeletal muscle. *Biol Rev Camb Philos Soc*. 84:143-59. doi: 10.1111/j.1469-185X.2008.00066.x
- Vandorpe DH, Small DL, Dabrowski AR, Morris CE. 1994. FMRFamide and membrane stretch as activators of the *Aplysia* S-channel. *Biophys J*. 66:46-58. doi: 10.1016/S0006-3495(94)80749-0
- Wang X, Engisch KL, Li Y, Pinter MJ, Cope TC, Rich MM. 2004. Decreased synaptic activity shifts the calcium dependence of release at the mammalian neuromuscular junction *in vivo*. *J. Neurosci*. 24:10687-10692. doi: 10.1523/JNEUROSCI.2755-04.2004
- Webb J, Wu FF, Cannon SC. 2009. Slow inactivation of the NaV1.4 sodium channel in mammalian cells is impeded by co-expression of the beta1 subunit. *Pflugers Arch*. 457:1253-1263. doi: 10.1007/s00424-008-0600-8
- Weber MA, Nielles-Vallespin S, Huttner HB, Wöhrle JC, Jurkat-Rott K, Lehmann-Horn F, Schad LR, Kauczor HU, Essig M, Meinck HM. 2006. Evaluation of patients with paramyotonia at ²³Na MR imaging during cold-induced weakness. *Radiology* 240:489-500. doi: 10.1148/radiol.2401050737
- Yu N, Morris CE, Joós B, Longtin A. 2012. Spontaneous excitation patterns computed for axons with injury-like impairments of sodium channels and Na/K pumps. *PLoS Comput Biol*. 8:e1002664. doi: 10.1371/journal.pcbi.1002664
- Yu JG, Liu JX, Carlsson L, Thornell LE, Stål PS. 2013. Re-evaluation of sarcolemma injury and muscle swelling in human skeletal muscles after eccentric exercise. *PLoS One* 8:e62056. doi: 10.1371/journal.pone.0062056
- Zhang Y, Hamill OP. 2000. On the discrepancy between whole-cell and membrane patch mechanosensitivity in *Xenopus* oocytes. *J Physiol*. 523:101-115. doi: /10.1111/j.1469-7793.2000.00101.x
- Zhang Y, Gao F, Popov VL, Wen JW, Hamill OP. 2000. Mechanically gated channel activity in cytoskeleton-deficient plasma membrane blebs and vesicles from *Xenopus* oocytes. *J Physiol*. 523:117-130. doi: 10.1111/j.1469-7793.2000.t01-1-00117.x
- Zhang B, Wang C, Wang H, Kong H, Gao F, Yang M, Zhang J. 2020. Feasibility of MRI based oxygenation imaging for the assessment of acute limb ischemia. *Ann Transl Med*. 8:315. doi:10.21037/atm.2020.02.139

Table 1 Pump-Leak/Donnan models for excitable cells

	Model name: Item:	SM-CD	WD-CD	CN-CD
1	<i>P-L/D model</i> <i>an “ion homeostatic unit”</i> <i>(i.e., same membrane C_m</i> <i>for each) depicting:---</i>	one “slice” of the many needed to comprise a multinucleate skeletal muscle fiber (explained in Figure 8C,D)	a counterfactual computational “tool” (a Pump-Leak dominated analog of SM-CD)	a roundish soma of a cortical neuron in brain-slice (Dijkstra et al 2016) (termed CN-CD here)
2	<i>in ancestral vertebrates</i>	SMFs >40% of body mass*	---	brain <0.2% of body mass*
3	<i>in humans</i>	SMFs ~40% of body mass (e.g. Rolfe et al 1997)	---	brain ~2% of body mass (Mink et al 1981)
4	<i>“electrophysiological life-style” served</i>	low excitability low duty-cycle, “off-on-off” AP signal	(same as SM-CD)	high excitability, high duty-cycle, complex nuanced V_m signalling
5	<i>dominant st-state fluxes</i>	ANIONS (Cl^-)	CATIONS (Na^+, K^+)	CATIONS (Na^+, K^+)
6	<i>....hence...TYPE of st-st ion homeostasis</i>	([big P_{Cl}] [small I_{Naleak}]) Donnan dominated	([small P_{Cl}] [big I_{Naleak}]) Pump-Leak Dominated	([small P_{Cl}] [big I_{Naleak}]) Pump-Leak dominated
7	<i>model complexity</i>	MINIMAL P-L D system	minimal (like SM-CD)	NON-minimal P-L D (has K/Cl co-transporter)**
8	<i>P_{Cl} (molecular identity)</i>	mostly ClC-1	----	unclear (Rungta 2015)
9	<i>P_K (molecular identity)</i>	girkS (DiFranco et al 2015), K-ATP, others (needs work)	----	diverse, including 2 pore domain K^+ channels
10	<i>P_{Na} (molecular identity)</i>	UNKNOWN	----	NALCN, in part

* The teleost bodyform is broadly similar to that of the extinct lobefin fishes close to the common ancestors of mammals and teleosts (see Casane and Laurenti, 2013) and so, finding no estimates for ancestral vertebrates, %-ranges here for them are conservatively gauged from data for extant adult teleosts (typically ~60% and ~0.15% of total body mass for skeletal muscle (Johnston et al 2011; Helfman et al 2009) and an extant lobefin, whose brain occupies <<0.15% of body mass (Dutel et al 2019).

The K/Cl cotransporter of CN-CD is eliminated in MN-CD (Minimal Neuron"-CD) for which $V_{rest} = E_{Cl}$; **Table 2 gives parameters and steady-state values for both. Steady-state comparisons show: the cotransporter strongly hyperpolarizes E_{Cl} , reduces Vol_{cell} and minimally affects $[K^+]_i$, $[Na^+]_i$, V_{rest} , ATP-consumption (as appropriate, the impacts of adding (e.g.) various Na^+ -transporters to SM-CD could similarly be probed).

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_{maxpump}	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 Some operational attributes of the P-L/D models and new queries

	Model name	SM-CD	WD-CD	CN-CD
	<i>P-L/D model depicts</i>	“slice” of multinucleate SMF (estimated to be ~1 myonuclear domain volume)	a <u>counterfactual</u> “tool”; P-L dominated analog of SM-CD	a roundish soma of a cortical neuron in brain-slice (Dijkstra et al 2016)
1	<i>ATP-consumption (or, relative to SM-CD)</i>	51.1 amol/s (1X)	180.4 amol/s (3.5X)	270.9 amol/s (5.3X)
2	<i>pump-reserve</i>	11.1-fold	3.1-fold	2.1-fold
3	<i>rate-limiting P for $\Delta V(t)$ as anoxic rundown starts</i>	P_{Na} (=0.3 $\mu\text{m}^3/\text{s}$)	P_{Cl} (=2.5 $\mu\text{m}^3/\text{s}$, and, at $t=0$, $V_m = E_{Cl}$)	P_{Cl} (=2.5 $\mu\text{m}^3/\text{s}$, and, at $t=0$, V_m positive to E_{Cl})
4	<i>time till ectopic firing during anoxic rundown</i>	22.7 minutes (rundown at: 10 mV per 12 min)	6 minutes (10 mV per 2.9 min)	<100 milliseconds (triggered by pump-off dep’n)
5	<i>no V-gated channels (*) -- 20 min into anoxic rundown: V_m, Vol_{cell}</i>	(SM*-CD) depolarized by 19 mV swollen 1.05-fold	---	(CN*-CD) depolarized by 45 mV swollen 1.4-fold
6	<i>saddle-node pump-strength & $[Na^+]_i$</i>	7.9% pump-strength, $[Na^+]_i = 88$ mM	26.5% pump-strength, $[Na^+]_i = 92$ mM	65% pump-strength, $[Na^+]_i = 42$
7	<i>patho-physio threshold pump-strength</i>	339% pump-strength ($V_{pathological} = -34$ mV)	----	185% pump-strength ($V_{pathological} = -37$ mV)
8	<i>examples of conditions to examine ion homeostatically</i>	hibernation onset/offset, compartment syndrome, delayed onset muscle soreness, Nav & other channelopathies, muscular dystrophies	(a WD-CD/SM hybrid) the “ P_{Cl} disease”, myotonia –mutant CLC-1 → excitable SMFs with [small P_{Cl}][small I_{NaLeak}]	ischemia, migraines, epilepsy (Dijkstra et al 2016), optimizing analgesic protocols for neuropathic pain (see Lorenzo et al 2020)
9	<i>examples of relevant physiological/biological/ evolutionary questions (re SM-CD and CN-CD)</i>	CLC-1 underlies [big P_{Cl}]. Does this channel’s very small unitary-g somehow benefit SMF ion homeostasis?	can smooth muscle (Lauf et al 2019) be modeled by modifying WD-CD (Ca^{2+} -gCl & gK, co-K/Cl)?	did early vertebrate hindbrain vascularization (Rahmat & Gilland 2014) prioritize fuelling of central neurons?

Table 4

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

ION HOMEOSTASIS : A PUMP-LEAK/DONNAN FEEDBACK PROCESS

FIGURE 1 Fundamentals. A. Ion homeostasis as an autonomous Pump-Leak/Donnan (P-L/D) feedback process with two sensor/effector mechanisms

P-L/D systems cannot sense deviations from their “set point” values. The otherwise blind-to-system-values $3\text{Na}^+/\text{2K}^+$ -pump functions as a sensor/effector by actively extruding Na^+ faster (thus hyperpolarizing V_m) when $[\text{Na}^+]_i$ is higher (as per Figure 9Ai,ii) while the (Donnan-effectors)/ P_{Cl} combination, via thermodynamics/electrostatics, functions as a sensor/effector that prevents osmo-imbalance and maintains compartment neutrality. (The weak V -dependence of Na/K -pumps (Rakowski et al 1997) is not modeled here).

B. Minimal P-L/D systems at steady-state Implied but not depicted: **1)** the membrane has an H_2O -permeable bilayer of capacitance, C_m , that can store charge until $\sim V_m \pm 200$ mV, **2)** compartments are neutral, osmo-balanced and iso-potential, **3)** the cellular surface-area-to-volume (SA:V) ratio handles normal swelling perturbations without bilayer-rupture, **4)** ATP is available ad libitum, **5)** average Donnan effector valence=-1, **6)** the exterior is infinite/invariant. V -gated channels are not depicted because normally they do not contribute to steady-state. Impermeant cytoplasmic anions (mostly proteins) act as Donnan effectors; Donnan effectors passively influence the transmembrane distributions of permeant ions and H_2O . Animal cells’ electrogenic $3\text{Na}^+_{\text{out}}/\text{2K}^+_{\text{in}}$ -ATPases cycle faster at $\uparrow [\text{Na}^+]_i$. ($[\text{K}^+]_e$ is invariant here, so pump sensitivity to $[\text{K}^+]_e$ is not modeled). At ion homeostatic steady-state, the hyperpolarizing pump-flux precisely counterbalances in/out fluxes across P_{Na} and P_{K} . For these minimal systems, $V_m = V_{\text{rest}} = E_{\text{Cl}}$ because in/out anion flux across P_{Cl} balances at V_{rest} . In minimal systems, moreover, the absolute quantity of Donnan effectors determines resting Vol_{cell} (Usher-smith et al 2009) (MN-CD vs CN-CD offers a simple minimal vs non-minimal comparison; see Table 1 footnotes(**)). For self-regulating P-L/D ion homeostasis, these are the minimal components. In the top and bottom minimal systems, $\text{P}_{\text{Na}}:\text{P}_{\text{K}}:\text{P}_{\text{Cl}}$ ratios differ, as does the steady-state pump-rate (implied by arrow-size). For the steady-state (net fluxes=0) at top, influx/efflux is predominantly anions (Cl^-) passively balanced by Donnan effectors, and at bottom, influx/efflux is predominantly cations actively counterbalanced by $[\text{Na}^+]_i$ -sensitive energy-consuming pump work. The top P-L/D ion homeostatic steady-state we describe as “Donnan dominated” ($[\text{big } \text{P}_{\text{Cl}}][\text{small } I_{\text{Naleak}}]$), the lower one as “Pump-Leak dominated” ($[\text{small } \text{P}_{\text{Cl}}][\text{big } I_{\text{Naleak}}]$).

FIGURE 2
The P-L/D model,
SM-CD, handling a real-
life ion homeostatic
stress-test

A. SM-CD undertaking a stress-test based on a rat soleus muscle experiment (Nielsen and Clausen 1997). SM-CD parameters are in **Tables 2**. H-H properties of the Nav and Kv channels are as depicted in **Figure 9C**, with rate constants as in **Table 4**. AP heights decline during the train. [Na⁺]_i rises, eliciting ↑ATP-consumption, ↑[Cl⁻]_i, ↑Vol_{cell} and (not shown) ↓[K⁺]_i. The expanded inset shows APs at ~5 seconds. When EPSCs stop, V_m hyperpolarizes and [Na⁺]_i decays steadily due to electrogenic Na⁺-

extrusion (hyperpolarizing I_{pump}). ATP-consumption, having ↑~7-fold (engaging ~2/3 of SM-CD's ~11-fold pump-reserve) remains high for ~40 seconds ("11-fold" refers to the maximal/resting ratio for ATP-consumption (i.e., 566/51.1; see **Figure 9A**). Na⁺-extrusion, (I_{pump}(t)) hyperpolarizes V_m until [Na⁺]_i returns to steady-state (**Table 2** gives models' steady-state values). During the AP train, SM-CD steadily swells though by <2.5%; depending on a SMF's geometry and mechanics, this might engage its caveolar tension-buffer (see Sinha et al 2011.; Morris 2018), a system not included in SM-CD. Once AChR, Nav, and Kv channel activity ceases, fluxes are strictly ion homeostatic. For ~2 min, [Cl⁻]_i and Vol_{cell} undershoot, then converge on steady-state. Though ΔVol_{cell} is small [Cl⁻]_i almost doubles; new techniques (DiFranco et al 2019) would make this measurable. SM-CD's very small P_{Na} ensures that the transiently-large I_{pump} is not undermined by a concurrently large I_{Naleak}. The passive ion homeostatic processes, obeying the constraints set by Donnan effector electrostatics/thermodynamics (rapidly, thanks to SM-CD's [big P_{Cl}]), entrain to the [Na⁺]_i-dependent Na⁺-pumping. H₂O permeability is high enough for swelling to be effectively instantaneous; very small driving forces on Cl⁻ explain why (in spite of [big P_{Cl}]) [Cl⁻]_i is slowest to converge back to steady-state. Vol_{cell}(t) mirrors [Cl⁻]_i(t). It would lag volume only if H₂O permeability was rate-limiting. **B.** For a single AP, precisely the same phenomena are seen; notice that the time scale for Vol_{cell} is the longest (not shown in this case, but identical to [Cl⁻]_i(t)). Notice the reminder (*) that ATP-consumption is directly proportional to (hyperpolarizing) I_{pump} (paired plots, **Figure 9A**). **Figure 6** provides further biophysical details.

FIGURE 3
Anoxic rundown in inexcitable neuron and SMF models

A. CN*-CD and **B.** SM*-CD are CN-CD and SM-CD with V-gated channels (gNav, gKv plus "gCl(V)" in CN-CD) zeroed. Systems (non-minimal CN*-CD, minimal SM*-CD) start at steady-state. In V_m panels, arrows highlight the ΔV_m at I_{pump} -off and I_{pump} -on. Pump-off depolarizes CN*-CD by ~8 mV, SM*-CD by <2 mV (for excitable CN-CD, the pump-off ΔV_m triggers APs; see **Figure 4A**). The SM*-CD rundown resembles Fig. 2 of Fraser and Huang (2004) (inexcitable amphibian SMF, $V_{rest}=-90$ mV, more complex pump formulation). At $t=0$, driving forces are small on Cl⁻ and K⁺, large on Na⁺. Current trajectories show fluxes through P_{Na} and P_K

(both bigger-valued in CN*-CD than in SM*-CD) dominating early rundown (though initially K⁺ feels the smaller driving force, $I_{Na,leak}$ through P_{Na} is the limiting cation flux). Swelling (Vol_{cell} tracks $[Cl^-]_i$) is checked far more effectively by SM*-CD's extremely small P_{Na} than by CN*-CD's [small P_{Cl}]; CN*-CD swells 1.4X in 20 min, while SM*-CD swells 1.4X in 76 min. At 105 min (at pump-on) $[Na^+]_i$ maximally activates (566 amol/s) pumps in CN*-CD. To reach the same loss of gradient as CN*-CD at 105 min, anoxic rundown in SM*-CD would need to be extended to 15.5 hrs. Mainly because of its greater rundown at 105 min, recovery is slower in CN*-CD. Both models also recover fully at pump-on when anoxia is imposed for a total of 272 minutes (not shown). If recovery had been started at the same membrane voltage in both systems, CN*-CD would still recover more slowly because of its larger P_{Na} .

FIGURE 4

Anoxic rundown of CN-CD and SM-CD

$V_m(t)$ panels in **A** and **B** include expanded insets; the main trace in **B** (SM-CD) flags several insets. The blue dot expansion box shows that SM-CD switch-off of hyperpolarizing I_{pump} causes a depolarizing “RC” response in contrast to CN-CD’s larger pump-off depolarization which (**A**, inset) triggers ectopic APs. The 50 mV scale, lower right inset, **B**, applies also for the upper right inset. **A**) switch-off of CN-CD’s large steady-state hyperpolarizing I_{pump} triggers ectopic APs; the

pump-off depolarization (readily evident in CN*-CD, **Figure 3A**) triggers ectopic APs that continue for <1 minute. Nav and Kv channels precipitously dissipate the cation gradients. This already-dire situation is exacerbated by the (pathological) opening at ~-20 mV, of a depolarization-activated Cl^- pathway (Rungta at a 2015; Dijkstra et al 2016). CN-CD continues swelling, degrading towards DE. **B**) In SM-CD, I_{pump} switch-off produces a ~2 mV RC response (as in **Figure 3**, where this is too fast to be temporally-resolved) then a very slow V_m rundown whose rate is limited by small P_{Na} . [Big P_{Cl}] lets E_{Cl} rapidly track V_m . Because $P_K \gg P_{Na}$, K^+ efflux mostly neutralizes Na^+ influx. Slight swelling reflects the extent of $[Na^+ + Cl^-]_i$ entry, osmo-balanced by H_2O influx (but the Cl^- driving force is negligible). Ectopic firing starts at 22.7 minutes. SM-CD Nav and Kv density is 3X greater than in CN-CD, so gradient dissipation is even more precipitous. As E_K decreases during APs, Na^+ influx is neutralized increasingly by Cl^- influx, and since the $[Na^+ + Cl^-]_i$ influx $\rightarrow$ $[Na^+ + Cl^- + H_2O]_i$ influx, Vol_{cell} suddenly increases. Ectopic firing tapers abruptly, the small V_m oscillations reflecting V-gating (SM-CD, pink triangle, V_m inset). When $[Na^+]_i$ and $[K^+]_i$ attain external values, V_m is still <0 mV and $[Cl^-]_i < [Cl^-]_e$ so net Cl^- entry continues, accompanied by H_2O for osmo-balance; compartment neutrality is retained during swelling (ongoing $\uparrow H_2O$) because $\uparrow [Cl^-]_i$ compensates for $\downarrow$ [impermanant anion] $_i$. The system would rupture before converging on DE (see **Table 1**).

FIGURE 5
WD-CD, a counterfactual
analog of SM-CD

To clarify how [big P_{Cl}] plays out in the context of SM-CD's other ion homeostatic elements (beyond its electrophysiological role of "big input conductance"), anoxic rundown is compared in WD-CD, a minimal P-L/D model with big P_K as "big input conductance". **A** ("control") is the SM-CD anoxic rundown of V_m (for other parameters, see **Figure 4B**). **B**, as labeled is the WD-CD anoxic rundown. WD-CD's ion homeostatic response to an EPSC-triggered AP is shown in **Figure 6**. WD-CD was subjected to the **Figure 2A** stress test (see **Figure Supp01C**).

FIGURE 6
Biophysics of
Donnan dominated
and
Pump-Leak dominated
systems

Ion homeostasis is scrutinized comparatively for 3 related minimal P-L/D systems: they are the normal version and an ischemic version (LA-CD; 30% pump-strength) of SM-CD, both of which are Donnan dominated systems, and SM-CD's Pump-Leak dominated counterfactual analog, WD-CD. In addition to the APs and subsequent ΔV_m , ATP-consumption (directly proportional to I_{pump}), $\Delta[ion]_i$ for (Na^+ , K^+ and Cl^-) and Vol_{cell} are plotted. In each system, ion homeostatic restoration to steady-state involves a volume oscillation commencing with AP-induced "H₂O blips" which are abrupt $\uparrow Vol_{cell}$ (grey arrow) whose amplitude reflects the magnitude of

excess Na^+ entry (i.e., the Na^+ -entry demanding osmo-balance because it is unaccompanied by K^+ -exit). This excess is greater in SM-CD and LA-CD (whose blips overlap) than in WD-CD (whose bigger P_K explains its smaller blip). The amplitude of the models' immediate Donnan-responses (see millisecond resolution trajectories for the $\uparrow [Cl^-]_i(t)$) depend on what fraction of the "electro-neutralization-of-excess- Na^+ -entry" task is achieved by Cl^- entry (for [big P_{Cl}] SM-CD and LA-CD, most of it; for [small P_{Cl}] WD-CD, very little of it). The AP perturbation ends in milliseconds but ion homeostatic feedback continues for minutes, the pump sensor/effector process actively extruding Na^+ (=hyperpolarizing I_{pump}) at a rate proportional to $[Na^+]_i$, while the passive Donnan sensor/effector feedback mechanism supports Cl^- and H₂O fluxes that keep in/out H₂O activities balanced and the cytoplasm electroneutral.

FIGURE 7

SM-CD bistability: bifurcation plots **Ai, Bi, Ci** are SM-CD bifurcation plots, as labeled (**Figure Supp 02A, B** has $[K^+]_i$ and $[Cl^-]_i$ bifurcation plots). Exit from the physiological continuum occurs at the “saddle-node” marked **X**; exit from the pathological continuum occurs at the unstable point marked **#**. Parts of **Bi** and **Ci** are expanded (**Bii, iii** and **Cii, iii**). **Aii** is the $V_m(t)$ trajectory for 100%→7.9% pump-strength (i.e. saddle-node); it takes ~10 hours for SM-CD to depolarize to firing threshold (inset expands ectopic burst) and then re-stabilize in a pathological steady-state. For bigger pump-strength drops, time-to-ectopic-firing falls sharply (inset plot), converging on the anoxic (0%) rundown time (22.7 minutes) (see **Figure 4A**). For patho-physiological comparisons, we recomputed the CN-CD (Dijkstra et al 2016) bifurcation plots (we report Vol_{cell} , not cross-sectional area); CN-CD’s threshold and normal steady-state points are included, as indicated. Although notional Donnan Equilibrium (**DE**) is marked in **Ai, Bi, Ci**, rupture would occur prior to attaining **DE** (as per **Ci**) unless bilayer area was augmented ($\uparrow C_m$).

Figure 8

Model building.

A. From CN-CD to SM-CD SM-CD was devised for direct comparison against CN-CD, an established cortical neuron model (Dijkstra et al 2016). Where possible the two are identical. Differences reflect the distinctive electrophysiological "lifestyles" of neurons (Dijkstra et al 2016) and SMFs (Fraser and Huang, 2004; Fu et al 2011; Ruff 2011; Pedersen et al 2011; Novak et al 2015). The Dijkstra et al (2016) model addressed cortical neurons in brain slice preparations. As an indicator of Vol_{cell} they reported confocally-imaged cross-sectional neuronal areas; we recalculated to obtain spherical volumes from those X-areas. **B,C,D. From CN-CD to SM-CD to SMF-like cytomorphology**

To foster comparisons, $\text{Vol}_{\text{cell}} = 2000 \mu\text{m}^3$ and $\text{SA} = 2000 \mu\text{m}^2$ from CN-CD (Dijkstra et al 2016) was used for SM-CD so there is excess SA beyond what is required to enclose a sphere of $2000 \mu\text{m}^3$, but neuron and SMF cytomorphologies differ, so **Bi,ii**, and **Ci,ii** sketches the disposition of the excess SA (as per **A**) for each. Additionally, **C** indicates that for SM-CD, the $2000 \mu\text{m}^3 \text{Vol}_{\text{cell}}$ would represent one cross-sectional slice "ion homeostatic unit" through a cylindrical multinucleate myofiber. The $2000 \mu\text{m}^2$ of sarcolemma would encircle, not enclose,

myoplasm. **Cii** is thus "SM-CD" as modeled here ($\text{Vol}_{\text{cell}} = 2000 \mu\text{m}^3$, $\text{SA} = 2000 \mu\text{m}^2$). The P-L/D models are not, however, 3-dimensional, so the difference noted in the excess SA for CN-CD and SM-CD does not affect computations here. However, as **Di** shows, for cylindrical geometry, $2000 \mu\text{m}^2$ of sarcolemma disposed as a smooth (no excess-SA) ring of SA could encircle vastly more than $\text{Vol}_{\text{cell}} = 2000 \mu\text{m}^3$, depending on slice width. For example, the $10 \mu\text{m}$ slice depicted in **Dii** would enclose almost $32,000 \mu\text{m}^3$ of myoplasm. Or, for an example with a little excess SA, $2000 \mu\text{m}^2$ could be disposed, say, as follows (not shown): a $15 \mu\text{m}$ radius cylinder of $20 \mu\text{m}$ "slice width" with a lateral $\text{SA} = 1885 \mu\text{m}^2$ encircling $14,137 \mu\text{m}^3$ with $115 \mu\text{m}^2$ added to bring the slice to $\text{SA} = 2000 \mu\text{m}^2$ and providing a small (1.06-fold) tension-buffering excess SA. Throughout the figure, linear, area and volume dimensions have units of μm , μm^2 and μm^3 respectively. Schematics are not to scale. For reference, Fraser and Huang (2004) modeled a $75 \mu\text{m}$ diameter fiber with $\text{SA}/\text{Vol}_{\text{cell}} = 5 \times 10^5 \text{ cm}^2/\text{liter}$; the **Dii** slice has $\text{SA}/\text{Vol}_{\text{cell}} = 6 \times 10^5 \text{ cm}^2/\text{liter}$ (for SM-CD as per **Cii**, $\text{SA}/\text{Vol}_{\text{cell}} = 25 \times 10^5 \text{ cm}^2/\text{liter}$).

Figure 9

Pump and Excitability.

A,B. The pump: ATP-consumption (or hyperpolarizing I_{pump}) as $[\text{Na}^+]_i$ varies

Ai. For the pump (Hamada et al 2003) used in all models here, ATP-consumption as a function of $[\text{Na}^+]_i$; normal pump-strength for CN-CD and SM-CD as indicated on two scales (0-30 mM, and inset, 0-160 mM). **Aii** replots the inset, but for hyperpolarizing I_{pump} , showing its direct proportionality to ATP-consumption. **Bi.** Using a logarithmic Y-axis, ischemia and pump up-regulation are depicted by scaling the 100% pump-strength curve as labeled. **Bii.** Lower left region **Bi**, expanded. Typically for enzyme kinetics plots, the abscissa is the independent variable ("Given $[\text{Na}^+]_i$, what is the ATP-consumption?"), but for P-L/D systems at steady-state,

the pump kinetics plot used "in reverse" gives a read-out of steady-state $[\text{Na}^+]_i$ as illustrated here for SM-CD and LA-CD. In the P-L/D systems, $-I_{\text{NaLeak}} = \text{hyperpolarizing } I_{\text{pump}}$ and so, via **Equation 19** a model's I_{NaLeak} converts to steady-state ATP-consumption. See dashed green line at 51.1 amol/s (SM-CD) and almost-overlapping dashed red line at 50.7 amol/s (LA-CD); where these intersect their models' pump-strength curve (100% for SM-CD; 30% for LA-CD), the X-co-ordinate (black dots, below) indicates steady-state $[\text{Na}^+]_i$. Likewise for CN-CD (X-axis crossing, magenta "crosshairs", **Ai** inset). **Ci,ii. Nav and Kv channels' electrical excitability in the P-L/D models** Hodgkin-Huxley (H-H) plots for the V-dependent channels used here, calculated using **Equation 7** and kinetic constants given in **Table 4**. The excitable (action potential competent) P-L/D models here provide explicit leak permeabilities (P_{Cl} , P_{K} and P_{Na}); there is, therefore, no non-specific "H-H gLeak". Also unlike standard H-H formulations, driving forces are all electrodiffusive (not ohmic) (see **Methods**, **Equations 2 and 3**).

Figure 10

Cation Permeabilities and V_{rest}

With SM-CD's other parameters held constant, **A** and **B** plot V_{rest} as P_{Na} or P_K vary (green dots, normal SM-CD V_{rest}) as they might, say, as a consequence of hormonal modulation. In **C**, plots **A** and **B** coalesce, giving V_{rest} as $P_{Na}:P_K$ varies. Green cross-hairs ($P_{Na}:P_K = 0.3$) indicate SM-CD normal V_{rest} (-86.0 mV) with other P-L/D models V_{rest} also indicated at the $P_{Na}:P_K$ appropriate for each model. The models vary

enormously in their PCI values, and CN-CD (but not MN-CD) has a K/Cl cotransporter that modifies E_{Cl} . Nevertheless, V_{rest} is overwhelmingly dominated by the $P_{Na}:P_K$ ratio. These calculations all assume normal pump strength. As per **Figure 7Ai**, increasing SM-CD pump-strength above the normal (100%) negligibly affects V_{rest} but if pump strength falls below ~20% V_{rest} depolarizes (due to diminished hyperpolarizing I_{pump} plus depolarizing I_{Nav} window current).

Supplemental Figures

Figure Sup 1

Stress-test A. By ~30 seconds after APs stop in SM-CD, ATP-consumption (Na^+ -extrusion) it almost back to steady-state when pump-strength=200% (maximum ATP-consumption = $2 \times 566 = 1,132$ amol/s or $I_{\text{pumpmax}} 2 \times 54.5 = 109$ pA) i.e., when pump-reserve=22.2-fold. **B.** $V_m(t)$ for SM-CD (pump-strength 100%) from **Figure 2A** but expanded to emphasize the effect of hyperpolarizing I_{pump} during ion

homeostatic recovery, post-1200 APs. **Ci.** For the same 1200 AP stress-test performed by SM-CD (**Figure 2A**), ATP-consumption by WD-CD. **Cii.** $V_m(t)$ for WD-CD expanded over the voltage range relevant to ion homeostatic recovery. At 10 seconds (immediately post-1200 APs) V_m goes to -88.5 mV (then, to -89.8 mV before returning to steady-state). Also at $t=10$ seconds, ATP-consumption peaks at 370 amol/s while (not shown), $[\text{Na}^+]_i=32$ mM, $[\text{Cl}^-]_i=5.75$ mM, and $\text{Vol}_{\text{cell}}=2004.5 \mu\text{m}^3$.

Figure Sup 2

Bifurcation

analysis A,B, from the same SM-CD bifurcation analysis as in **Figure 7A,B,C** plots for steady-state $[\text{K}^+]_i$ and $[\text{Cl}^-]_i$. **Ci,** the SM-CD V_m bifurcation plot showing the location of #, the unstable threshold on the pathophysiological steady-state continuum. The equivalent bifurcation point in CN-CD is called a Hopf bifurcation by Dijkstra

et al (2016). **ii,iii** At $t=0$, SM-CD (on the pathological continuum) pump-strength is stepped from 338% to 339%. The resulting trajectory is plotted (**ii**), with a portion expanded (**iii**).